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AFTER-RIPENING AND GERMINATION OF SEEDS OF TILIA, SAMBUCUS, AND RUBUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 247

R. C. ROSE

Introduction

This paper gives the results of an attempt to determine the conditions favoring the after-ripening and germination of the seeds of *Tilia americana*, *Sambucus canadensis*, and *Rubus Idaeus*, and some of the chemical processes involved therein. Since layering of these seeds usually results in very low percentages of germination, it was thought possible to discover some other means of overcoming their dormancy.

Literature

The present state of our knowledge of the causes of delay in germination, and the means of overcoming it, is admirably summarized in a recent paper by CROCKER (5). He divides seeds which show delay in germination into 7 classes. In 3 of these the seed coats play the important rôle, while in the fourth dormancy is occasioned by the embryo. Where dormancy or poor germination is due to the seed coat, the use of concentrated sulphuric acid as a carbonizing agent has become a common practice. ROSE (23) mentions ROSTRUP (24) as the first to resort to this treatment, and lists TODARO (25), HILTNER (12), JARZYMOWSKI (14), BOLLEY (2), and LOVE and LEIGHTY (19) as investigators applying the same method. EWART (9) found this treatment effective with several

species of *Acacia*, as did CROCKER (unpublished work) with *Scirpus*. The length of time required by this treatment varies from a few minues to several hours, depending upon the resistance of the coats.

Boiling water or warm water, used as a forcing agent, has proved effective in a number of cases where hard-coatedness is the cause of the delay. BRUYNING (3), working with the seeds of *Ulex europaeus*, found that a treatment of 1–5 seconds with boiling water raised the percentage of germination from 13 for untreated seeds to 53·5–75·5 for treated seeds. HONING (13) obtained his best results with *Albizzia* seeds by soaking them in water at 60° C. for at least 3 hours, while with *Mimosa* 60–70° C. proved most effective, as did 70–75° C. for *Pithecolobium*. Soaking seeds of *Crotalaria* in warm water proved disadvantageous. BOLLEY (2) states that improvement in germination was obtained by this method if the exposure was not long enough to kill the embryo.

NOBBE (21) mentions ALEXANDER VON HUMBOLDT as the first investigator to use chemicals as forcing agents. From the time of HUMBOLDT (1793) up to 1873, the date of publication of NOBBE's book, many investigators used as forcing agents a great variety of substances, both organic and inorganic. The range of substances used is more interesting than the results obtained. Moreover, quickly germinating seeds were used and in such cases the effect of forcing agents is not so striking as where dormancy is involved. Of the more recent workers in this field, LEHMANN (16) was the first to emphasize the importance of chemical substances in connection with germination. He showed that the seeds of *Ranunculus sceleratus* are forced into germination by Knop's solution, by soil, by soil wet with weak solutions of hydrochloric acid, potassium hydroxide, ferric chloride, and hydrogen peroxide. Two years later GASSNER (10) found Knop's solution effective on unthreshed seeds of *Chloris ciliata*, and more recently (11) has shown that for several other seeds various nitrogen compounds, especially nitrites and nitrates, are effective forcing agents. *Chloris ciliata* was found to have a membrane impermeable to potassium nitrate and magnesium nitrate, and from this GASSNER concludes that the effect is upon the seed coat alone. LEHMANN (17) and LEHMANN and OTTENWÄLDER (18), working with seeds representing

a number of different families, showed that acids in low concentrations, especially hydrochloric acid, are effective forcing agents. CROCKER and DAVIS (6) obtained similar results for seeds of *Amaranthus* (unpublished work) and *Alisma*. Bases are equally effective for *Sagittaria* and *Alisma*, but not for *Amaranthus*. According to OTTENWÄLDER (22) bases exert an inhibitory effect on seeds of *Epilobium hirsutum*.

In those cases where a state of dormancy exists in the embryo itself (*Crataegus* and *Malus*), temperatures slightly above freezing have been found effective in hastening after-ripening (7). In *Crataegus*, as ECKERSON (8) has shown, the hypocotyl becomes more acid as after-ripening progresses; hence dilute acids hasten after-ripening by acting upon the hypocotyl directly.

Material

The seeds used in these experiments were gathered in the summer or the fall of 1916 and 1917. Each year those of *Sambucus* were all collected on the same day from neighboring plants. *Tilia* seeds of the 1916 crop were collected during October from trees growing on the dunes at the southern end of Lake Michigan. The 1917 crop was gathered during September from trees in the parks of Washington, D.C. The seeds of *Rubus* were collected during late June 1916 from neighboring plants of several varieties, but no attempt was made to keep those of the different varieties separate. Among the seeds of all 3 species were found many without embryos or with defective embryos. In most cases this fact accounts for the varying number of seeds used in the cultures. Approximately 60 per cent of *Rubus*, 75 per cent of the 1916 *Sambucus*, and 80 per cent of the 1916 crop of *Tilia* were viable. Not more than 5 per cent of the 1917 crop of *Tilia* and *Sambucus* were defective.

Histology and microchemistry of seed coats

SAMBUCUS: *Endocarp.*—The seed in cross-section shows in the lignified endocarp 3 regions: (1) the outermost, consisting of 3 or 4 layers of cells of irregular size and shape, with thin walls and large lumina; (2) a middle one of 1 or 2 layers of fibers in cross-section; and (3) an inner one of 1 or 2 layers of fibers in longitudinal section. *Seed coat.*—This consists of several layers of collapsed cells with

lignified walls; the cells contain a considerable quantity of reducing sugar.

TILIA: *Pericarp.*—This is composed of two layers: (1) a surface region of loose fibers with cellulose walls, and (2) a thicker region of lignified fibers. *Seed coat.*—This consists of 3 regions: (1) cells with suberized or cutinized walls; (2) one layer of palisade cells with (a) outer end walls of cellulose, (b) a lignified light zone, (c) a pectinized region, and (d) a lignified region; and (3) 3 or 4 layers of cells with walls which stain with ruthenium red and give the ceric acid test.

RUBUS: *Endocarp.*—This consists of 2 layers: (1) an outer layer, variable in thickness, of lignified fibers longitudinally arranged in cross-section of the fruit; (2) an inner region of 4 or 5 layers of lignified fibers transversely arranged in cross-section of the fruit. *Testa.*—This consists of 4 regions: (1) 1 layer of cushion-shaped cells with lignified walls; (2) 4 layers of collapsed cells with cellulose walls; (3) 1 layer of collapsed cells with thick pectinized walls; and (4) 1 layer of cells with cellulose walls which appear as a thickened outer wall of the endosperm.

Microchemistry

In table I are given the results of the microchemical tests made upon the endosperm and embryo of each of the kinds of seeds used. Owing to the lack of a sufficient number of germinating seeds of *Sambucus* several of the tests have not been completed. The storage materials in all the seeds are very similar, starch, fats, and protein being found in every case. In addition to these *Sambucus* contains amylodextrin. *Tilia* contains much more fat and phytosterol than either *Sambucus* or *Rubus*. The phytosterol shows up as a bright red layer around the fat globules when sections of the seeds are placed in concentrated sulphuric acid. Oxidase is present in the dry seeds in very small quantities, and in the germinating seeds benzidine gives a positive test only after several hours. Peroxidase, while present in dry *Tilia* seeds, is much more abundant in the germinating seeds. Dry seeds of *Sambucus* and *Rubus* give no peroxidase reaction. Catalase is found in both dry and germinating seeds of all 3 species.

TABLE I

Substance	Tilia				Sambucus				Rubus			
	Ungerminated		Germinated		Ungerminated		Germinated		Ungerminated		Germinated	
	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo
Starch	+	++	++	+++	−	++	−	++	−	−	+	++
Amylodextrin	−	−	−	−	++	−	+	−	−	−	−	−
Reducing sugar	−	−	+	++	−	−	?	?	−	−	+	+
Protein reaction:												
Xanthoprotein	+	+	+	++	++	++	?	?	+	+	+	+
Biuret	++	++	++	++	+	+	?	?	++	++	+	+
Fat	+++	++	++	+	+	+	+	+	+	+	+	+
Phytosterol	+++	++	++	+	+	+	?	?	++	++	+	+
Tannin	−	−	−	−	−	−	−	−	++	+	++	+
Oxidase	−	−	+	++	−	−	?	?	−	−	+	+
Peroxidase	+	+	++	++	−	−	+	++	−	−	+	++
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Reaction	Neutral or acid	Neutral or acid	Alkaline	Acid	Alkaline	Alkaline	Alkaline	Acid	Acid	Acid	Acid	Acid

Conclusive determinations in regard to the reaction of fresh dry seeds of *Tilia* have not been made, but preliminary tests, where neutral red was used as an indicator, indicate that the endosperm and cotyledons are acid and the hypocotyl alkaline. Seeds kept in dry warm storage for 9 months show an acid reaction throughout. Hydrogen ion determinations, the data for which are given later, showed an acid reaction for the stored seed as well as for the germinating ones. As germination begins, the reaction of the embryo of *Sambucus* changes from alkaline to acid, but the endosperm remains alkaline. Both dry and germinating *Rubus* seeds are acid. A qualitative analysis of the ash of *Tilia* seeds showed iron, calcium, magnesium, potassium, and aluminium present. No tests were made for sodium.

Experimental data

Freshly harvested *Tilia* seeds with a moisture content of 10 per cent or less, or seeds kept in dry warm storage for several months, fail to germinate when placed on a moist substratum and kept at room temperature. This is true not only of seeds with coats intact, but for those with the coats chipped or entirely removed. Fungi and bacteria soon attack seeds with the coats broken and decay takes place in a few days. The percentage of water held by air-dry seeds is shown in table II. The seeds used for these determinations were dried in a partial vacuum at 80° C. until the weight was constant.

TABLE II

WATER CONTENT OF AIR-DRY *Tilia* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Water loss in gm.	Percentage of water loss
Coats off	1.2754*	0.0788	6.17
Coats on	1.8559	0.1782	9.60
Coats on	2.1904	0.1574	7.18
Coats on	1.5024	0.1130	7.52

*Average of 4 duplicates.

The variations in the percentage of water lost by the seeds with coats on is due to the presence of seed coats which contained no endosperm and embryo. That the failure of air-dry *Tilia* seeds,

coats either on or off, to germinate is not due to an inability to absorb water is indicated by table III. The data given in this table were obtained by soaking seeds in distilled water at room temperature until they had come to constant weight. Here again the

TABLE III

WATER-HOLDING CAPACITY OF AIR-DRY *Tilia* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Water absorbed in gm.	Percentage of water absorbed
Coats off.....	1.2848*	1.2071	93.95
Coats on.....	2.1540	0.7841	36.40
Coats on.....	1.7842	0.4146	23.24
Coats on.....	2.1198	0.4804	22.66
Coats chipped.....	1.4963	1.5020	100.38
Coats chipped.....	1.5040	1.5717	104.50
Coats chipped.....	1.9590	1.9185	97.93

*Average of 4 duplicates.

variations in the percentage of water absorbed are in part due to the presence of seed coats which contain no endosperm and embryo. Even with the coats chipped it is not always possible to eliminate all empty coats or defective seeds. The fact that the coats interfere with water absorption to a considerable extent is clearly shown in the table. The fact that seeds with coats removed or chipped, however, and with a moisture content approximately equal to their air-dry weight will not germinate when placed on a moist substratum at room temperature, is sufficient proof that water absorption is not the only limiting factor to growth.

That seeds that have been stored in the air-dry condition when the seed coats are intact can be forced to germinate is shown by the following experiment. Approximately 7000 seeds (200 gm.) of the 1916 crop, with pericarps removed and coats chipped, were placed on moist cotton in large Petri dishes and kept at 4–6° C. from March 24, 1917, to June 10, 1917, a total of 78 days. At the end of that time and before being transferred to a higher temperature, several hundreds showed the hypocotyl protruding from the endosperm for 1.5–2.5 cm. Of these, 100 were planted in soil in the greenhouse and 71 per cent produced seedlings. A second lot of 100 seeds was planted in soil out of doors, and 64 per cent

produced seedlings. Two lots of 500 each were selected from the seeds in which the hypocotyl was still inclosed within the endosperm. These were planted in soil in the greenhouse and in the garden and gave 20 and 25 per cent germination respectively. All seeds not planted were again placed in cold storage. Twelve days later 400 with hypocotyls protruding from the endosperm were planted in soil in the greenhouse. Of these, 348, or 87 per cent, produced seedlings within a week. By July 24, 1666 of these 7000 cold storage seeds had germinated at a low temperature. Of the ungerminated seeds 100 placed on moist cotton at room temperature gave 31 per cent germination in one week. The roots of these were short and thick and showed a great tendency to coil. At the same time air-dry seeds which had been stored at room temperature, when placed in soil or on moist cotton, decayed. Seeds kept in cold storage showed for the first few days a great tendency to mold, so that it was necessary to sterilize them with a 3 per cent solution of hydrogen peroxide for 1 hour on two separate occasions. With longer storage an immunity toward fungi is established, and although the coats may be covered with a thick layer of mycelia the endosperm and embryo are not attacked. Sections of the seeds examined under the microscope failed to show any hyphae present within the living tissue. On November 6, 1917, 6 cultures of 50 seeds each of both the 1916 and 1917 crops were placed in moist storage at 0–2° C., where they were allowed to remain for 140 days. At the end of that time no germination had taken place, which is in direct contrast with the result obtained in 1916 with seeds stored at 4–6° C. The failure to obtain germination here is interpreted as being due to the use of too low a temperature. The assumption that the exposure to this temperature was too long will hardly explain the results obtained, since if the temperature were not too low germination should begin as soon as the after-ripening process is complete. The results given in table IV, showing the percentage of germination obtained when these seeds were transferred to a temperature of 10–12° C., indicate that the storage temperature and not the length of exposure to it is the limiting factor.

This conclusion is strengthened further by the following experiment. Unfortunately no count of the number of seeds germinated

was made, as the experiment was used primarily for a different purpose. Approximately 1000 seeds of each of the 2 crops, stored under the same conditions as those indicated in table IV, showed no

TABLE IV

SEEDS OF *Tilia* STORED AT 0–2°C. FOR 140 DAYS; THEN AT 10–12°C.

NUMBER OF CULTURE	PERCENTAGE OF GERMINATION AFTER			
	12 days at 10–12° C.		19 days at 10–12° C.	
	1916 seeds	1917 seeds	1916 seeds	1917 seeds
1	66	24	74	28
2	68	20	72	24
3	70	12	80	18
4	74	34	82	34
5	70	26	76	30
6	19	22	56	30

germination after 140 days at a low temperature. When brought to the higher temperature the 1916 seeds germinated vigorously and in large numbers for the first 12 days and until the hypocotyls were 2–3 cm. long. From this point on no development took place and the seedlings gradually died. Here a temperature of 10–12° C. seems to be too low for continued growth. The 1917 seeds germinated much less vigorously, in fewer numbers, and only a few developed hypocotyls 2 cm. long. Comparing the results obtained in 1916 with those obtained in 1917, it is seen that the seeds after-ripen and germinate at temperatures slightly above freezing. DAVIS and ROSE (7) working with *Crataegus* found that after-ripening takes place most rapidly at 3–6° C., and that temperatures considerably higher are more favorable for germination and growth. At 0–2° C. *Tilia* seeds after-ripen but do not germinate. At 4–6° C. after-ripening and germination both take place, the latter taking considerable time. After-ripened seeds germinate poorly at room temperature. Once germination has begun at the low temperature, growth is best at temperatures above 12° C. The germination of *Tilia* seeds depends, therefore, upon the proper regulation of the temperature, and can be accomplished by a period of after-ripening in moist storage at 0–2° C., followed by a sojourn of 2 or 3 weeks at 10–12° C. until germination is well under way,

and finally by a transfer to a still higher temperature in order to permit vigorous growth. These conclusions are drawn from the facts that (1) seeds after-ripened at 0–2° C. did not germinate until transferred to a temperature of 10–12° C.; (2) although germination began at the higher temperature, growth soon ceased; and (3) seeds which had been after-ripened and which had begun to germinate at 4–6° C. grew well when transferred to soil in the greenhouse. Table IV suggests that one-year old seeds are better than fresh, but additional data upon this point are desirable. A nurseryman with many years' experience in the growing of trees and shrubs states that if *Tilia* seeds are allowed to become dry between the time of maturing and the time of layering a low percentage of germination results. On the other hand, if a high moisture content is maintained during this period no difficulty in germination is encountered. Up to the present time the author has been unable to obtain seeds which at the time of gathering had a moisture content of more than 10 per cent, and it seems probable that the water content of *Tilia* seeds is generally low at harvest time. While these seeds do not after-ripen to any considerable degree in air-dry storage, those that have been in the air-dry condition for a year after-ripen perfectly when put in a moist germinator at a low temperature. There seems to be no injury, therefore, even from protracted air-dry storage. No discussion is necessary to show that field conditions are not those most favorable for the obtaining of high percentages of germination. Neither does the nurseryman, when layering seeds, control the temperatures to the extent necessary to secure maximum results.

HYDROGEN ION CONCENTRATION.—The determinations of the hydrogen ion concentrations were made with the hydrogen electrode. Twenty seeds were pulverized in a mortar, and, except in instances to be noted later, 25 cc. of water added. The temperature varied from 27 to 33° C., but in every case the necessary correction was made. The determinations were made upon the seeds in the unafter-ripened condition, after-ripened but not germinated, with hypocotyl 2 mm. to 5 mm. long, and with hypocotyl 0.5 cm. to 2 cm. long.

ECKERSON (8) has already shown that the acidity of the hypocotyl of *Crataegus* increases as after-ripening progresses. Her

determinations were made by the titration method with phenophthalein as an indicator. The P_H of seeds with the hypocotyls 0.5 cm. to 2 cm. long is approximately 4 times as great as that of the unafter-ripened seeds. While this is not as great an increase as that found by ECKERSON, it may be due to the fact that her determinations were made upon the dormant organ alone, while here the whole seed was used, or to differences between the two kinds of seeds. Determinations made by the titration method would also probably give values much higher than those obtained by the hydrogen elctrode.

Table V shows that the weight of the seeds increases as after-ripening progresses. This is not due to an increase in dry weight,

TABLE V

CONCENTRATION OF HYDROGEN ION OF *Tilia* SEEDS IN DIFFERENT STAGES OF AFTER-RIPENING

Condition of seeds	Weight in gm.	P_H
1 Air-dry	0.384	2.24×10^{-7}
2 Air-dry	0.443	2.00×10^{-7}
3 Air-dry	0.379	2.58×10^{-7}
4 Air-dry	0.379	2.40×10^{-7}
5 After-ripened	0.714	3.24×10^{-7}
6 After-ripened	0.752	3.02×10^{-7}
7 With hypocotyls 5 mm.	0.943	6.76×10^{-7}
8 With hypocotyls 5 mm.	0.932	5.75×10^{-7}
9 With hypocotyls 5 mm.	0.946	7.59×10^{-7}
10 With hypocotyls 0.5–2 cm.	1.553	1.18×10^{-6}
11 With hypocotyls 0.5–2 cm.	1.638	1.10×10^{-6}
12*With hypocotyls 0.5–2 cm.	1.578	9.33×10^{-7}
13*With hypocotyls 0.5–2 cm.	1.709	9.33×10^{-7}
14†With hypocotyls 0.5–2 cm.	1.450	1.05×10^{-6}
15†With hypocotyls 0.5–2 cm.	1.571	1.05×10^{-6}
16 After-ripened at room temperature (10 days)	0.689	2.51×10^{-7}
17 After-ripened at room temperature (10 days)	0.642	2.51×10^{-7}

* 50 cc. of water used.

† 100 cc. of water used; 25 cc. of water used for all others.

since no photosynthesis has taken place, but to the large amount of water absorbed. ECKERSON (8) likewise observed an increased water-holding capacity for the hypocotyl of *Crataegus* as after-ripening progressed. Of greater significance in this connection is the fact that, at least for the most advanced stage of after-ripening, variations in the amount of water used with the sample had little

effect upon the hydrogen ion concentration. With samples 10 and 11, 25 cc. of water were used, with samples 12 and 13, 50 cc., and with samples 14 and 15, 100 cc. Although the variation of P_H is considerable, it is by no means as great as that of the amount of water used, nor is it in the same direction. That the degree of dilution has no effect upon the P_H suggests the presence of buffer salts, formed by the action of fatty acids produced during germination and the constituents of the ash already mentioned.

After-ripened seeds similar to those used in samples 5 and 6, which had failed to germinate when kept at room temperature for 10 days, gave a P_H corresponding very closely to that shown by unafter-ripened seeds. This suggests that after-ripening is a reversible process, a fact to which CROCKER (5) has called attention, and that a decrease in acidity may lead to secondary dormancy.

TITRATABLE ACID.—Determinations of the titratable acid were made upon dry, after-ripened, and germinating seeds. For each determination the seeds were ground in a mortar with 10 cc. of water and titrated with N/10 sodium hydroxide with phenophthalein as an indicator. Titrations were made with freshly prepared samples and with others which had been allowed to stand for 48 hours. To the latter were added 10 drops of toluol and 0.5 cc. of N/10 hydrochloric acid. Table VI shows the number of cubic centimeters of N/10 sodium hydroxide necessary to neutralize the free acid in each sample. The figures are the average of duplicate determinations. Corrections have been made for the acid added.

TABLE VI

Condition of seeds	Fresh samples	After 48 hours	Percentage of increase
Dry	0.41	0.87	112.2
After-ripened	0.45	1.87	315.5
Germinating	1.18	2.80	137.2

While the amount of acid present is greatest in germinating seeds, it is seen that after autodigesting 48 hours the greatest percentage of increase over the freshly prepared samples is in seeds well after-ripened. Here is shown the fact that the after-ripened

seeds have a great power of increasing their alkali absorption, which may be due to lipase activity.

CATALASE.—Determinations of catalase activity of dry, after-ripened, and germinating seeds were made by means of APPLEMAN'S apparatus (1). The samples, ground in a mortar, were all reduced to the same degree of fineness by rubbing them through bolting cloth. The catalase determinations were made at 25° C. To 5 cc. of water containing 0.02 gm. of pulverized seed material was added 5 cc. of Oakland dioxygen and the amount of oxygen released was measured after 1, 2, 3, and 5 minutes of activity. APPLEMAN has pointed out that small amounts of acid greatly reduce or entirely destroy catalase activity. In order to remove this possible source of error the Oakland dioxygen used was neutralized by the addition of N/10 NaOH, or an excess of $CaCO_3$ was added to the meal. The data given in table VII are the averages of duplicate determinations. They show that dry, after-ripened, and germinating seeds, in the order named, exhibit increasing catalase activity. ECKERSON (8), employing microchemical methods, arrived at similar conclusions for seeds of *Crataegus*.

TABLE VII

CONDITION OF SEEDS	REACTION OF REAGENT	OXYGEN IN CC. LIBERATED AFTER			
		1 minute	2 minutes	3 minutes	5 minutes
1. Dry seeds	Neutralized*	2.5	4.2	5.25	7.0
2. After-ripened (dried 2 days)	Neutralized*	7.1	11.8	15.4	21.5
3. After-ripened (dried 2 days)	With $CaCO_3$	6.8	11.9	14.8	21.05
4. After-ripened (not dried)	With $CaCO_3$	6.75	11.3	15.05	20.75
5. Germinating	With $CaCO_3$	19.4	27.86	31.5	37.03

*0.80 cc. N/10 NaOH to neutralize 25 cc. dioxygen.

Drying after-ripened seeds for 2 days at room temperature has no effect on the amount of oxygen liberated, as is shown by comparison of samples 3 and 4.

Further evidence for the effect of the acid of the dioxygen upon catalase activity is shown in table VIII. Determinations made with after-ripened seeds not dried and with germinating seeds gave similar results. A comparison of the last 2 determinations show

that the neutralization of dioxygen or the addition of $CaCO_3$ is sufficient to eliminate any error due to the acidity of the reagent or the meal.

TABLE VIII

EFFECT OF REACTION OF SOLUTION UPON AMOUNT OF OXYGEN LIBERATED FROM DIOXYGEN BY *Tilia* SEEDS

CONDITION OF SEEDS	REACTION OF REAGENT	OXYGEN IN CC. LIBERATED AFTER			
		1 minute	2 minutes	3 minutes	5 minutes
After-ripened (dried 2 days)....	Not neutralized	2.1	3.1	3.6	4.3
After-ripened (dried 2 days)....	Neutralized	7.1	11.8	15.4	21.5
After-ripened (dried 2 days)....	With $CaCO_3$	6.8	11.9	14.8	21.05

OXIDASE ACTIVITY.—The determinations of oxidase activity were made on dry, after-ripened, and germinating seeds in BUNZELL'S (4) simplified apparatus with pyrogallol as the reagent. The material used, except in the case of the dried seeds, had been after-ripened at 0–2° C. for 140 days and then kept at 10–12° C. until a large percentage of the seeds had begun to germinate. After being dried in a vacuum over lime for 3 days at room temperature the seeds were ground in a mortar and the determinations made on 0.02 gm. of meal. Table IX shows the readings in centimeters of mercury after 3 hours and after 20.5 hours.

TABLE IX

OXIDASE ACTIVITY OF DRY, AFTER-RIPENED, AND GERMINATING SEEDS OF *Tilia*

Time	Dry seeds	After-ripened	Hypocotyls 1–5 mm. long	Hypocotyls 0.5–2 cm. long
After 3 hours.....	0.52	1.03	2.01	1.39
After 3 hours.....	0.53	1.10	1.92	1.52
After 20.5 hours.....	0.67	1.52	2.57	2.42
After 20.5 hours.....	0.68	1.67	2.52	2.07

During the experiment the temperature averaged 31.3° C. with a variation of ±0.1 of a degree. Variations in the volume of air in the tubes due to this slight variation in temperature have been corrected by means of check tubes containing water only. The

results show that the oxidase activity rises with after-ripening and germination. Once germination has begun, no increase is to be noted.

DISCUSSION.—The results obtained show that the dormancy exhibited by the seeds of *Tilia* is not due to any property of the seed coat, although that structure may serve to lengthen the dormant period, but is to be ascribed to conditions obtaining within the endosperm or the embryo or both. In this respect *Tilia* resembles *Crataegus*, and the conditions necessary for after-ripening and germinating of the former are very similar to those required by the latter. Even with these conditions well known and various differences between dormant and after-ripened seeds clearly shown, it is still impossible to define the term after-ripening in anything more than general terms. The similarity of *Tilia* and *Crataegus*, with respect to the conditions necessary for after-ripening, does not permit one to conclude that the process in the two is the same. In any case after-ripening is not to be attributed to a change in any one condition, but to a series of changes which may vary for each individual case. Dormancy is to be looked upon, perhaps, as a condition of equilibrium in a series of chemical reactions; after-ripening as a displacement of this condition. Why low temperatures are effective in causing these changes and why the range of effective temperatures is so narrow are questions still to be answered.

SAMBUCUS

KINZEL (15) states that for *Sambucus nigra* freezing for .2 winters is sufficient to bring only 39 per cent of the seeds to germination. Even longer freezing is necessary for the seeds of *S. racemosus*. Results obtained by the writer in experiments to be described are very similar to those given by KINZEL, and show that in neither case have the conditions necessary for germination been even approximately determined.

Nurserymen claim that layering results in almost perfect germination if the seeds are not allowed to become dry between the time of maturing and the time of layering. Air-dry seeds are considered worthless. These statements are in a large measure

confirmed by the following experiments, although sufficient data are not yet available to warrant a final statement.

Seeds removed from berries and allowed to dry at room temperature for 2 days failed to germinate within 2 weeks when placed on moist cotton, although they never contained less than 22 per cent of moisture. Fresh seeds on moist cotton kept at 4–6°, 0–2°, or 8° C. have never given more than 20 per cent germination when placed at room temperature or above. Air-dry seeds have given no better results. Although these seeds were kept at the low temperature for not less than 2 months, a longer period may be necessary. The experiments show that failure to germinate is not entirely due to injury resulting from drying, although that may be one of the determining factors. Neither is it to be attributed to inability of air-dry seeds to absorb water, since the quantity taken up in 48 hours by seeds with coats intact is equal to 38.55 per cent of their air-dry weight, while seeds with coats punctured absorb 39.16 per cent. Air-dry seeds contain approximately 6 per cent of water.

The effect of layering is shown by the following experiments, in which the number of seeds used for the 1916 crop was 1000 and for the 1917 crop 5000. Two lots of air-dry seeds of the 1916 crop were mixed with soil. One lot was kept at 15–20° C., the other out of doors over winter. In spring the percentages of germination were 8 and 44 respectively. Fresh seed of the 1917 crop, which had not been permitted to become dry when treated in the same way, gave 51 per cent and 77 per cent respectively. Air-dry seeds of the 1916 crop one year old failed to show any germination. Loss of water seems to be accompanied by a reduction in vitality.

Air-dry seeds gathered on October 14, 1916, were treated within 30 days with weak solutions of a large number of acids, bases, and salts. The acids used were malic, citric, tartaric, acetic, and butyric; the bases, potassium hydroxide, ammonium hydroxide, and sodium hydroxide; and the salts, sodium sulphate, nickel sulphate, ammonium sulphate, zinc sulphate, potassium sulphate, potassium nitrate, sodium nitrate, cobalt nitrate, ammonium nitrate, calcium chloride, sodium chloride, barium chloride, and potassium thiocyanate. The dilutions of the acids were N/200

and N/400; of the bases, N/1000, N/2500, N/5000, and N/10,000; and of the salts, N/20 and N/200. The number of perfect seeds in the cultures varied from 43 to 96. In only 4 cases was the number below 60, and the average was 75. This variation is due to the presence of empty seed coats which could not be distinguished from the perfect seeds until they had taken up a considerable quantity of water. It was later found possible to candle the seeds and thus eliminate the majority of the empty coats. The candling was done by means of an incandescent light supported below a glass plate upon which the seeds were placed. Between the light and the plate was placed a vessel of water to prevent undue heating. The seeds were placed in 20 cc. test tubes containing the solutions and allowed to soak for 24 hours. At the end of that time the solutions were drawn off and the seeds distributed over the moist walls of the test tubes, which were then plugged with cotton and kept at a temperature varying from 4 to 23° C. As soon as the seeds began to show signs of germination, they were removed from the tubes and placed in Petri dishes on moist cotton and kept at room temperature. Germination was slow, in the majority of cases extending over a period of 3 months. In the case of acetic acid, N/400, 58 per cent of the seeds germinated at the end of 176 days. The acids other than acetic showed little effect. The length of time over which bases can have any effect must be short, since in dilute solutions they are soon neutralized by the carbon dioxide of the air and that produced by the seeds. The cultures which showed germinations equal to or better than the checks are listed in table X.

In order to test the effect of constant low temperature upon seeds soaked in solution of various chemicals, a second set of cultures was prepared in the manner already described and kept at 4–6° C. for 63 days. At the end of that time the tubes were placed at room temperature. To the list of substances used in the preceding experiment were added potassium citrate, potassium tartrate, potassium acetate, potassium chlorate, ammonium nitrate, potassium iodide, lithium chloride, ammonium chloride, magnesium chloride, sodium nitrite, and dipotassium phosphate, and also hydrochloric acid and sulphuric acid. The concentrations of the mineral acids were N/1000, N/2500, N/5000, N/10,000, and of the

salts N/20, N/200, and N/1000. Germination began 4 days after the cultures were placed at room temperature and continued for 18 days. At the end of that time in practically all of the cultures, in addition to the seeds which had germinated, others were found

TABLE X

Sambucus SEEDS IN DILUTIONS OF ACIDS, BASES, AND SALTS; TEMPERATURE 4–23° C.

Substance	Normality of solution	Number of seeds	Percentage of germination
Distilled water	.....	78	12
Distilled water	.....	85	10
Distilled water	.....	68	10
Distilled water	.....	83	13
Acetic acid	N/200	79	18
Acetic acid	N/400	72	28
Malic acid	N/400	75	15
NH_4OH	N/2500	77	18
NaOH	N/1000	88	19
NaOH	N/2500	70	17
$(NH_4)_2SO_4$	N/20	75	28
$(NH_4)_2SO_4$	N/200	67	15
$ZnSO_4$	N/20	50	22
KNO_3	N/200	80	30
$NaNO_3$	N/20	46	19
$NaNO_3$	N/200	72	30
$CoNO_3$	N/200	59	71
KCNS	N/200	66	31

with the seed coat ruptured, but showing no sign of growth. All cultures in which a forcing effect of the solution is indicated by the germination of 20 per cent or more of the seeds are listed in table XI.

Out of 13 other substances not given in the table, 5 showed results equal to or better than the average of the checks in at least one dilution. The nitrates and sulphates are again found among the more effective substances. So far as the nitrogen compounds are concerned, these results agree with those of GASSNER (10) for seeds of *Chloris ciliata*.

Potassium nitrate, mercuric chloride, and potassium iodide used in connection with alternating temperatures had even less forcing effect than the substances given in table XI. The concentrations used were for potassium nitrate N/20, N/100, N/200, N/500,

N/1000, N/2000; for mercuric chloride N/400, N/1000, N/2000, N/4000, N/10,000; and for potassium iodide N/20, N/100, N/500, N/1000, N/2000. Three sets of cultures were set up in duplicate.

TABLE XI

Sambucus SEEDS IN ACIDS, BASES, AND SALTS

Substance	Normality of solution	Number of seeds	Percentage of germination	Percentage of seeds with ruptured coats	Total percentage of seeds affected
HCl	N/5000	65	17	6	23
H_2SO_4	N/2500	66	18	10	28
H_2SO_4	N/10,000	63	15	6	21
NH_4OH	N/1000	89	23	16	39
NH_4OH	N/5000	96	13	8	21
$C_6H_8O_9$	N/400	101	21	4	25
KNO_3	N/20	78	28	25	53
KNO_3	N/200	77	13	18	31
KNO_3	N/1000	86	24	8	32
$CoNO_3$	N/200	92	4	44	48
$CoNO_3$	N/1000	87	24	23	47
NH_4NO_3	N/200	108	14	39	53
NH_4NO_3	N/1000	56	14	12	26
$NaNO_3$	N/20	96	20	18	38
$NaNO_3$	N/200	83	33	20	53
$NaNO_2$	N/200	72	25	26	51
$NaNO_2$	N/1000	65	9	18	27
Na_2SO_4	N/200	94	10	10	20
$NiSO_4$	N/20	84	10	10	20
$NiSO_4$	N/200	86	7	34	41
$NiSO_4$	N/1000	76	15	10	25
$(NH_4)_2SO_4$	N/200	85	5	37	42
$ZnSO_4$	N/20	86	41	1	42
KCL	N/20	80	0	22	22
LiCl	N/1000	80	0	22	22
NaCl	N/200	89	1	37	38
NH_4Cl	N/20	97	2	24	26
NH_4Cl	N/200	84	13	15	28
Potassium citrate	N/20	84	0	21	21
Potassium citrate	N/1000	95	7	37	44
$KClO_3$	N/1000	81	9	18	27
KI	N/20	89	10	33	43
KCNS	N/200	90	9	12	21
K_2HPO_4	N/20	83	1	24	25
K_2HPO_4	N/1000	55	0	34	34
Distilled water	.....	70	0	7	7
Distilled water	.....	85	8	7	17
Distilled water	.....	109	1	14	15

One set was kept at 20° C. and a second at 30° C. The third set was kept at 20° C. for 18 hours and at 30° C. for 6 out of every 24 hours. The air in the tubes was changed every second day. The

duration of the experiment was 38 days. At the end of that time the only germinations obtained were those in the potassium nitrate, and in no case did these exceed 4 per cent. The seeds in the stronger mercuric chloride solutions were killed.

The rôle played by the coat in the behavior of the seeds has not been determined. Of naked embryos placed on moist cotton 32 per cent developed chlorophyll within a week, formed the hypocotyl arch, and attained a length of 5–10 mm. Naked embryos previously soaked in dilutions of hydrochloric acid and butyric acid and then placed on moist cotton gave no better results.

Seeds treated with concentrated sulphuric acid for 4–60 minutes and then kept under various conditions in regard to light, temperature, and oxygen pressure have never given over 20 per cent germination. A slight forcing effect by low concentrations of sulphuric acid was observed on seeds previously treated with concentrated sulphuric acid for 2–14 minutes and kept in the light at room temperature. Seeds immersed for 5 minutes in water at 40° C. in 55 days gave 25 per cent germination. Reheated at the same temperature for 3 minutes, 33 per cent germinated after 40 days. Longer heating at 40° C. or up to 70° C. gave lower percentages of germination. Untreated seeds gave no germination in the same length of time.

These results emphasize the following facts concerning the conditions necessary for the germination of *Sambucus* seeds: (1) air-dry seeds with a moisture content of 6 per cent or fresh seeds with a moisture content of 22 per cent will not germinate when placed on a moist substratum at room temperature; (2) this is not due entirely to injury resulting from drying, although that may be one of the determining factors; (3) air-dry seeds are able to absorb water to the extent of approximately 40 per cent of their air-dry weight, indicating that failure to germinate is not due to lack of water; (4) the effect of chemicals upon air-dry seeds is not marked, a slight forcing effect of several acids, bases, and salts has been observed, among which substances are found nitrates and sulphates; (5) the rôle played by the coat in the behavior of the seed has not been fully determined; (6) a slight forcing effect by low concentrations of sulphuric acid and by water at 40° C. has been observed;

(7) seeds remaining in contact with moist soil out of doors over winter gave 77 per cent of germination the next spring; whether this result is due to the low temperature, to certain constituents of the soil, or to a combination of these or other factors one cannot say.

The results obtained by KINZEL (15), together with those just summarized, show that as yet the conditions necessary for the germination of *Sambucus* seeds are not fully determined. To permit the water content of the seeds to fall below an undetermined critical point may lessen their viability. However, that some other condition or combination of conditions is responsible for the low percentages of germination must not be overlooked. KINZEL'S suggestion that prolonged freezing is necessary should be given due consideration.

RUBUS

Seed fruits of *Rubus Idaeus*, like the seeds of the 2 species already discussed, fail to germinate when placed on a moist substratum. It was determined that this is not due to an immature condition of the embryo. If the pericarp is left intact all treatments with low concentrations of acids, bases, and salts, immersion in warm water, cold storage, exposure to increased oxygen pressure, or to ether vapor, freezing and thawing, and injection with water under pressure are ineffective.

When buried in the soil at 15–20° C. or out of doors over winter, a low percentage of germination takes place if the seeds are kept moist. Two lots of 720 viable seeds buried for 140 days under these conditions gave respectively 40 per cent and 20 per cent germination. Of 2 similar lots of seeds buried in tightly stoppered bottles, one at constant, the other at varying temperatures, none germinated when planted in the soil in the greenhouse. That these results are not due to injury resulting from drying or to inability to absorb water is indicated in table XII. The removal of the endocarp was accomplished by soaking the seeds in concentrated sulphuric acid for approximately 2 hours. Following this treatment the seeds were washed quickly in a large amount of running water to prevent heating, then immersed in a 5 per cent solution of sodium bicarbonate to neutralize the remaining acid,

and finally rinsed in running water for 15 or 20 minutes. The carbonized endocarp was removed by rubbing the treated seed on filter paper. The selection of perfect seeds was now an easy matter.

Table XII shows that the water-absorbing power for the seeds with the endocarp removed is 36–37 per cent of their air-dry weight, while that for the seeds with the endocarp intact reaches

TABLE XII

WATER CONTENT AND WATER HOLDING CAPACITY OF *Rubus* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Weight of seeds dried *in vacuum* at 75° C.	Percentage of water *in air-dried* seeds	Weight of soaked seeds in gm.	Water absorbed by air-dry seeds in gm.	Percentage of water absorbed by air-dry seeds
Endocarp removed .	0.6686	0.5842	12.62*	0.9120	0.2434	36.40*
Endocarp removed .	0.6864	0.6009	12.45	0.9414	0.2550	37.15
Endocarp intact....	1.0464	0.9328	10.85	1.5053	0.4589	43.85
Endocarp intact....	2.0960	1.8680	10.87	3.0080	0.9120	43.51

* On basis of air-dry weight.

almost 44 per cent of their air-dry weight. From this it follows that the water absorbing power of the endocarp is greater than that of the seed with the endocarp removed. There is no evidence to show that the endocarp possesses any structure which would prevent the water absorbed by it from being passed on to the seed. Although seeds with the endocarp intact will not germinate, when that structure is removed by means of the sulphuric acid treatment germination takes place within a few days, as is shown in table XIII.

The greater amount of the germination takes place between the fourth and tenth days. In seeds germinating after the tenth or twelfth day, growth is usually slow and the seedlings are weak. Failure to secure 100 per cent germination is due to the fact that during the removal of the carbonized endocarp in almost every case the seed coat is ruptured and the endosperm exposed to the attacks of bacteria and fungi. With the carbonized endocarp intact, uncertainty as to the extent to which the acid had penetrated and the inability to determine the number of fruits containing viable embryos lead to greater error than that occasioned by the attacks of the bacteria and fungi.

MÜLLER (20) has recently pointed out that in various seeds that germinate readily the outward pressure of the contents at the time of rupture was but slightly greater than the breaking strength of the water-saturated coat, and CROCKER and DAVIS (6) have found that seeds of *Alisma* are held in a dormant condition because the force of the expanding contents is not sufficient to rupture the coats.

TABLE XIII

SEEDS OF *Rubus Idaeus* WITH ENDOCARP REMOVED; 100 SEEDS PER CULTURE; TEMPERATURE 18–23° C.

TREATED WITH ACID	PERCENTAGE OF GERMINATION AFTER				
	4 days	6 days	8 days	10 days	20 days
May 4	.....	.....	70	84	96
May 13	2	45	52	63	70
May 13	32	48	53	61	61
May 13	24	46	.....	55	55
May 24	.....	50	73	83	88
May 24	.....	20	63	77	84
May 24	.....	22	61	.....	80
May 24	.....	40	78	86	88
May 24*	.....	46	78	86	93
May 24*	.....	57	77	82	89
June 9	.....	50	.....	86	95

* In darkness.

Failure to absorb water is not the limiting factor, since both reach saturation after about 5 hours' soaking. Two facts indicate that *Rubus* seeds belong in the same class with *Alisma*. In the first place they germinate readily once the endocarp is removed, and in the second place even with the endocarp intact they absorb water readily. Occasionally ungerminated seeds with the endocarp removed have been found which when examined closely show no break in the coat. This suggests that the inner pectinized layer of the coat may play a part in the delay, either by limiting water or oxygen absorption, or both. As already indicated, the removal of the carbonized endocarp resulted in the rupture of the coat in practically 100 per cent of the seeds. This renders extremely difficult the determination of the part played by that structure.

Table XIV shows that the substrata most favorable for germination of naked seed are cotton, filter paper, and quartz sand. An

inhibitory effect is shown by garden soil, clay, and greenhouse soil, the effect of the last named being greatest. These soils acidified gave no better results. Calcium carbonate used on filter paper or in sand to neutralize any acid present in the medium or remaining on the seeds after the sulphuric acid treatment had no inhibitory effect. Glass wool moistened with a boiling water extract or a cold water extract of greenhouse soil cut down the percentage of germination to less than 50. Moreover, the seedlings were weak, with enlarged and discolored roots. In many cases germination started, but the roots were killed as soon as they came in contact with the substratum. Bone meal had been added to the greenhouse soil and this probably accounts for the injurious effect of the soil and the extracts. As is shown in table XIV, soaking in water for 24 hours

TABLE XIV

EFFECT OF SUBSTRATUM UPON GERMINATION OF NAKED SEEDS OF *Rubus Idaeus*; 100 SEEDS PER CULTURE; TEMPERATURE 18–23° C.

SUBSTRATUM	PERCENTAGE OF GERMINATION AFTER				
	6 days	8 days	10 days	12 days	25 days
Filter paper	50	77	86	90	95
Filter paper with $CaCO_3$	31	84	87	90	92
Quartz sand	48	80	83	85	92
Quartz sand	42	83	88	89	93
Quartz sand with 5 per cent $CaCO_3$	43	78	80	.....	88
Greenhouse soil	.....	.....	10	13	25
Greenhouse soil	.....	.....	.....	1	1
Greenhouse soil, acid	.....	.....	.....	1	1
Hot water extract greenhouse soil	4	22	41	.....	43
Cold water extract greenhouse soil	5	25	48	.....	48
Garden soil	5	25	.....	29	29
Garden soil, acid	1	9	.....	10	10
Clay	.....	3	9	11	19

previous to planting in garden soil raises the percentage of germination to 55. On the other hand, soaked seeds planted on moist cotton gave 71 per cent as against 72 per cent for unsoaked seeds. Germination was at practically the same rate in the two cases. Seeds planted on 5 per cent agar gave almost as high percentages as those on 1 per cent. The results given in tables XIV and XV show that the water supply is not the limiting factor.

Seeds in the soil are more exposed to attacks by fungi than those on agar or cotton. Previous soaking shortens the time the seeds must lie in the soil before germination begins, and hence lessens the chance for infection. Unsoaked seeds placed on moist cotton

TABLE XV

EFFECT OF WATER SUPPLY UPON GERMINATION OF SEEDS OF *Rubus Idaeus;* 100 SEEDS PER CULTURE; TEMPERATURE 20–25° C.

SUBSTRATUM	PERCENTAGE OF GERMINATION AFTER				
	6 days	8 days	10 days	12 days	16 days
1 per cent agar*	37	49	70	70	70
1 per cent agar*	22	50	.....	62	62
2 per cent agar*	30	37	50	50	50
2 per cent agar*	40	64	.....	73	73
5 per cent agar*	35	42	60	.....	60
5 per cent agar*	32	52	.....	61	61
Soaked 24 hours, then in garden soil	12	36	47	50	55
Soaked 24 hours, then on moist cotton	43	64	70	71	71
Not soaked, on moist cotton	32	66	72	.....	72

* Average of 2 duplicate determinations.

absorb water easily, hence swell more rapidly than in the soil, and moreover are less liable to infection. Under these conditions soaking offers no advantage.

Summary

GENERAL.—Air-dry seeds of *Tilia americana, Sambucus canadensis*, and *Rubus Idaeus* do not germinate when placed on a moist substratum at room temperature. In no case does water absorption seem to be the limiting factor. Air-dry seeds planted in the soil over winter give low percentages of germination.

TILIA.—Seed coats are not the cause of dormancy, although they may serve to lengthen the dormant period. A state of dormancy exists in the endosperm or embryo, or both.

Seeds with coats removed after-ripen at temperatures slightly above freezing. At 0–2° C. seeds after-ripen, but do not germinate. At 4–6° C. both after-ripening and germination take place. Seeds after-ripened at 0–2° C. germinate readily at 10–12° C., but very poorly at room temperature. Once germination has begun growth proceeds best at temperatures above 12° C.

As after-ripening progresses the hydrogen ion concentration increases, as do also the water holding capacity and the oxidase and catalase activities.

The greatest amount of free acid is present in the germinating seeds. Autodigestion of pulverized seeds shows the greatest acid increase in the after-ripened ungerminated seeds. This is probably due to their high lipase activity.

SAMBUCUS.—As high as 77 per cent of germination was obtained by layering fresh seeds out of doors over winter.

No satisfactory forcing agent has yet been found. A slight forcing effect of several acids, bases, and salts has been observed. The best of these forcing agents are nitrates and sulphates.

Although *Sambucus* seeds are probably injured by drying, that is not the only factor to be considered, since freshly gathered seeds with a moisture content of 22 per cent will not germinate when placed on a moist substratum.

As yet it has been impossible to approximate perfect germination, and much still remains to be learned concerning the conditions necessary to reach it.

RUBUS.—Dormancy is probably due to the high breaking strength of the endocarp. Seeds treated with concentrated sulphuric acid for 2 hours, then thoroughly washed, germinate readily on cotton, filter paper, or quartz sand.

The optimum temperature for germination lies between 20° and 25° C. Seeds germinate equally well in light or darkness. Naked seeds germinate poorly in soil. This may be due to the action of fungi, bacteria, or to other causes as yet unknown.

As a practical method for the germination of *Rubus* seeds, if one is not to resort to layering, the writer suggests the following: The seeds should be removed from the pulp as completely as possible. If the berries are crushed and then thrown into water most of the pulp can be floated off. The pulp still clinging to the seeds may be removed by allowing fermentation in water to take place or by treating the seeds with a 5 per cent solution of sodium hydroxide for 15–20 minutes, after which they should be thoroughly washed in running water. It is essential to dry the seeds for at least 24 hours, or the treatment with concentrated sulphuric acid which follows

will result in heating. The seeds should be left in the acid for approximately 2 hours.

In order to obtain uniform results it is advisable to use a large excess of acid and to prevent the seeds from gathering in clumps or layers. Frequent stirring is essential. By rubbing a few of the seeds in the palm of the hand from time to time it is possible to determine when the entire endocarp on a majority of the seeds has been carbonized. When this point is reached the acid should be drained away and the seeds thrown into an excess of cold water. It is advisable to change the water frequently or to put the seeds in running water, where they should be left for at least 15 minutes. When they are removed from the water they should be treated with an excess of a 5 per cent solution of sodium bicarbonate until bubbles cease to rise, after which they may be washed in running water for 15 minutes.

In order to remove the carbonized endocarp the seeds may be placed on filter paper and rubbed under the fingers. It is impossible to remove the endocarp if it has been allowed to become dry following the last washing.

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MISSOURI STATE FRUIT EXPERIMENT STATION
MOUNTAIN GROVE, MO.

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NOTES ON AMERICAN WILLOWS. IV

SPECIES AND VARIETIES OF SECTION LONGIFOLIAE

CAMILLO SCHNEIDER

In my paper on Mexican willows (BOT. GAZ. **65**:22. 1918) I have already dealt with some species of this well marked and entirely American section. In this article I intend to discuss all the members of this interesting group, which is, as M. S. BEBB (1891) and W. W. ROWLEE (1900) rightly stated, clearly defined from the other sections of the genus in both the New and the Old World. ANDERSSON (1858) was the first to recognize the close relationship of species like *S. sessilifolia* Nutt., *S. Hindsiana* Benth., and *S. taxifolia* Kth. to *S. longifolia* Muhl. Unfortunately he misunderstood most of the species described by NUTTALL, and therefore he did not give, even in 1868, a proper analysis of the forms of this section. In 1900 W. W. ROWLEE (Bull. Torr. Bot. Club **27**:247) made an attempt to rehabilitate all of NUTTALL'S species, and described several new species and varieties from the southwest, especially from California. His interpretation of NUTTALL'S species, however, is not free from grave errors owing to the lack of sufficient type material. Later C. V. PIPER studied those types of NUTTALL which are preserved in the British Museum, and communicated his notes to C. R. BALL, who in 1915 (BOT. GAZ. **60**:49) was able to identify *S. sessilifolia* and *S. fluviatilis* Nutt. I have not seen the types in the British Museum, but I have photographs of NUTTALL'S specimens of *S. exigua*, *S. macrostachya*, and *S. melanopsis* from the Herbarium of the Academy of Science at Philadelphia. Besides this I have also examined a few of NUTTALL'S willows at the Gray Herbarium, which also contains some cotypes of forms described by ANDERSSON. Photographs and fragments of ANDERSSON'S types from the Hookerian Herbarium at Kew are now in possession of the Arnold Arboretum, and Professor W. W. ROWLEE kindly sent me the types of his new species and forms so far as they are preserved in the Herbarium of Cornell

University. I wish to acknowledge here his courteous assistance, and to give the same acknowledgment to the curators of the Herbarium of the Geological Survey of Canada at Ottawa, of the Gray Herbarium, of the Herbarium of the Royal Gardens at Kew, of the Missouri Botanical Garden, of the New York Botanical Garden, of Stanford University, and of the U.S. National Herbarium for the loan of material representing the forms under discussion. For further material I am indebted to Miss ALICE EASTWOOD, San Francisco, California, Professor J. K. HENRY, Vancouver, B.C., Professor W. L. JEPSON, Berkeley, California, Mr. I. M. JOHNSTON, Upland, California, and Mr. J. C. NELSON, Salem, Oregon. I have also been able to go over the material of the Bebb Herbarium at the Field Museum, and am under obligation to Dr. C. F. MILLSPAUGH for what he has done to further my studies.

Sect. LONGIFOLIAE Andersson in Öfv. K. Vet.-Akad. Förh. **15**: 116. 1858; for further literature see SCHNEIDER in BOT. GAZ. **65**: 22. 1918.—Frutices mediocres (rariter parvi) vel alti arboresque, ramis densis caespitosis, cortice cinereo vel pl.m. brunnescente, ramulis elongatis virgatis brunneis vel purpureo-brunneis interdum nitidulis. Folia linearia, lanceolata, vel elliptico-oblonga, denticulata vel integerrima, nervis lateralibus satis distantibus, petiolis vulgo satis brevibus, stipulis saepe deficientibus vulgo parvis lanceolatis denticulatis. Amenta serotina vel primaria coetanea, pl.m. pedunculata vel ramos laterales normaliter foliatos saepe satis longos terminantia, singula vel ad 2–3 aggregata, pl.m. cylindrica, rarius ovalia; bracteae concolores, flavescentes, deciduae; flores masculi vulgo biglandulosi, diandri, filamentis liberis pilosis; feminei fere semper uniglandulosi, stylis nullis vel brevibus, stigmatibus bifidis laciniis linearibus vel brevibus; ovaria fructusve pilosi vel glabri, subsessiles vel pedicello glandulam usque duplo (rarius magis) superante instructi.

As already stated, the LONGIFOLIAE is an entirely American group, of which *S. taxifolia* var. *microphylla* ranges as far south as Guatemala, while a form of *S. longifolia* almost reaches the Arctic Circle in the Yukon Territory. From west to east the range of the group extends from the shores of the Pacific to those of the Atlantic, but it is not represented in southeastern United States from central

Virginia to Alabama and Florida. The center of its development is from California to Washington, Montana, and Texas.

Among the American willows the LONGIFOLIAE occupy an isolated position, and of the willows of the Old World it is difficult to say which can be taken for the nearest relatives of this group. I shall discuss this point later, and I can now only repeat that probably the forms of the sect. ALBAE Borr. might be regarded as rather closely related genetically to the LONGIFOLIAE.

In the following key it is recognized[1] that there are two rather well marked types in the group based on the form of the stigmas. In one, represented by *S. taxifolia* and *S. sessilifolia*, the lobes of the stigma are narrow and elongated, and in the older flowers mostly more or less revolute; while in the other group, the types of which are *S. exigua* and *S. longifolia*, the lobes are shorter and broader, not linear-lanceolate, the whole stigma often being quasi capitate. In some forms of *S. longifolia*, especially of var. *Wheeleri* from the northeast, the shape of the stigmas is rather intermediate. In the first group *S. taxifolia* is well distinguished from *S. sessilifolia* and its relatives by the short small aments, the small more or less globose anthers, and the small linear leaves; while *S. sessilifolia* and its varieties and *S. fluviatilis* have long cylindric aments, oblong-ellipsoid anthers, and longer, broader leaves. In the second group it is more difficult to separate the species because the main characters, glabrousness or pubescence of the ovaries and leaves, are more liable to variation. *S. melanopsis* with var. *Bolanderiana* represents a rather well marked type with glabrous ovaries, but in *S. exigua* as well as in *S. longifolia* we meet with forms of which the ovaries vary from densely pubescent to entirely glabrous. The

[1] It seems to be of interest to quote BEBB'S opinion as to the possibility of a taxonomic arrangement of the forms of this section (BOT. GAZ. 16:104. 1891): "Clearly marked as are the outer limits of the group it presents no lines of cleavage within by which it can be satisfactorily divided. No natural characters are found to coincide with such assumed distinctions, for instance, the 'linear lobes of the stigma,' made prominent in the attempt to separate *S. sessilifolia*. Each portion after subdivision remains as heterogeneous as was before the aggregate group. It may be possible, by emphasizing first one character and then another, as these are found to predominate in the different forms, to designate a number of subspecies and varieties; but so bewildering and intangible is the reticulated intergrading that the difficulty of segregation seems only to be heightened by every fresh acquisition of the material."

pubescence of the leaves too is very changeable, and only in connection with other characters can it be used to separate certain species and varieties.

Clavis specierum

Amenta brevia, mascula 5–13 mm. longa et circ. 8 mm. crassa, feminea satis pauciflora, fructifera haud ultra 2:1.2 cm. magna; antherae minimae pl.m. globosae vel subglobosae, haud vel paullo longiores quam latae; stigmatum lobi lineares vel lineari-lanceolati, vulgo 4–6plo longiores quam lati; stylus nullus vel subnullus; ovaria sessilia vel brevissime pedicellata; bracteae vulgo satis late obovato-rhombicae, pl.m. acutae, praesertim extus satis dense villosae; folia minima vel parva, linearia vel lineari-lanceolata, 10–30:1.5–3.5 mm. magna, subtus semper pl.m. sericea, margine breviter denticulata vel subintegerrima . . . . 1. *S. taxifolia*

Amenta longiora vel antherae ellipticae, circ. 1½–2plo longiores quam latae vel folia majora.

Stigmatum lobi lineares vel lineari-lanceolati, elongati, vulgo 4–5plo longiores quam lati, adulti pl.m. revoluti, stylo satis distincto iis breviore vel brevissimo fere semper bifido suffulti vel pl.m. sessiles; ovaria (saltem juniora) distincte sericea vel sericeo-villosula; folia novella semper utrinque pl.m. dense sericea vel sericeo-villosa.

Ramuli hornotini dense, etiam annotini pl.m. sericeo-villosi vel tomentelli; folia etiam adulta utrinque concoloria, canescentia, canoviridia vel viridescentia, semper pl.m. sericea vel sericeo-villosa, nervis primariss vix vel non visibilibus; ovaria semper satis dense sericeo-pilosa, sessilia vel subsessilia, pedicello fructuum quam glandula plus quam 2plo breviore; bracteae rarius extus versus apicem glabrescentes (confer etiam 4. *S. Parishianam*).

Folia ramulorum fertilium lineari- vel anguste lanceolata, fere semper distincte integerrima, etiam majora vix ad 8 mm. lata, apice pl.m. sensim acuminata, basi acuta, in petiolum satis distinctum attenuata, stipulis fere semper nullis, vel folia maxima majora, 6–8 cm. longa et ultra 8 mm. lata; amenta mascula 1.5–3 cm. longa et 5–6 (rarius 8) mm.

crassa, feminea fructifera 2–4 (–6): 0.8–1 cm. magna, ovaria (fructusque) sessilia vel subsessilia.

Folia fere semper lineari- vel anguste lanceolata et vulgo integerrima, stigmata semper satis elongata et pl.m. revoluta..... 2b. *S. sessilifolia* var. *Hindsiana*

Folia fere semper remote denticulata, interdum late lanceolata; stigmata breviora, paullo curvata et magis sessilia..... 2c. *S. sessilifolia* var. *leucodendroides*

Folia ramulorum fertilium (anguste vel) late lanceolata vel elliptico-lanceolata, majora 8–15(–17) mm. lata, saepe (saltem ad apicem) pl.m. distincte subspinuloso-denticulata, interdum paene sessilia, stipulis saepe pl.m. evolutis; amenta mascula 3–4.5 cm. longa et circ. 7 mm. crassa, feminea fructifera 4–6(–10) cm.: 8–10 mm. magna, ovaria (fructusque) subsessilia vel brevissime pedicellata

2. *S. sessilifolia*

Ramuli tantum novelli satis dense sericeo-tomentelli, jam hornotini glabrescentes vel glabriusculi vel folia adultiora satis glabra subdiscoloria, vel ovaria fructusque glabri vel subglabri (confer etiam var. *Wheeleri* sub 8. *S. longifolia*).

Folia anguste lanceolata ellipticave, interdum oblanceolata, apice pl.m. acuminata, basi acuta, distincte petiolata, stipulis saepe evolutis, adultiora superne intense viridia, subtus interdum subglaucescentia, satis glabrescentia vel tenuissime sericeo-pilosa, nervis etiam secundariis utrinque pl.m. visibilibus, ramulorum fertilium 7–14 mm. lata; ovaria initio pl.m. sericea vel sericeo-villosa, matura vulgo fere tota glabrescentia, subsessilia, pedicello fructuum glandula sicca interdum subaequilongo, bracteae fere semper extus versus apicem glabrescentes interdum basi excepta glabra..... 3. *S. fluviatilis*

Folia anguste linearia ad lineari-lanceolata, 1.5–5 (–8) mm. lata, utrinque pl.m. dense adpresse sericea; ovaria pl.m. sericea vel fere glabra, fructus partim pilosi vel glabri sed pedicello brevissimo vulgo piloso..... 4. *S. Parishiana*

Stigmatum lobi lanceolati vel elliptici, satis breves, saepissime 2–3plo longiores quam lati, adulti ut videtur nunquam distincte

revoluti, stylo nullo vel brevissimo non bifido suffulti, ovaria sericea vel glabra, subsessilia vel fructus pedicello glandulam interdum duplo superante instructi; folia ramulorum fertilium pl.m. dense sericea vel glabra.

Flores feminei glandulis 2 (dorsali interdum minima) instructi
6c. *S. exigua* var. *nevadensis*

Flores feminei glandula tantum ventrali instructi.

Glandulae florum masculorum 2 (ventralis et dorsalis).

Ovaria etiam juvenilia glaberrima.

Folia tantum valde juvenilia pl.m. distincte sericea vel ab initio pl.m. glabra vel tenuiter pilosa pilis saepe tantum sub lente visibilibus, utrinque concoloria vel superne viridia, subtus pallidiora, saepe pl.m. glaucescentia, nervis lateralibus secundariisque pl.m. prominulis.

Amenta fructifera valde densa, fructibus condensis breviter conicis pedicello subnullo vel satis brevi glandulam vix superante instructis, bracteae florum vulgo satis obovatae et truncatae; folia subtus fere semper pl.m. pallidiora vel glaucescentia, ramulorum sterilium satis late vel elliptico-lanceolata vel oblanceolata, rarius lineari-lanceolata.

Fructus 4.5–5.5 mm. longi (pedicello brevi excluso), amenta fructifera circ. 8–9 mm. crassa; folia ramulorum fertilium 3:0.4 ad 8:1.2, interdum ad 6.5:1.5 cm. magna, citissime glabrescentia vel pilis difficile visibilibus praedita (rarius initio satis dense adpresse argyraceo-sericea), satis distanter et breviter denticulata vel pl.m. integerrima; ramuli hornotini vulgo cito glabrescentes . 7. *S. melanopsis*

Fructus ad 6.5 mm. longi, amenta fructifera ad 1.2 cm. crassa; folia ramulorum fertilium ad 9:1.5 vel 17:1.7 cm. longa vel distinctius pilosa et denticulata vel ramuli hornotini magis pilosi
7b. *S. melanopsis* var. *Bolanderiana*

Amenta fructifera satis laxiflora fructibus separatis vel ovariis fructibusque longius conico-rostratis et pedicello distincto glandulam saepe duplo superante

instructis; bracteae florum vulgo oblongiores acutioresque; folia utrinque concoloria, pl.m. linearilanceolata vel linearia vel anguste lanceolata et satis distincte subdensius denticulata.

Fructus vix ultra 6 mm. (pedicello excluso) longi; folia anguste linearia, 2–4 mm. lata, venis lateralibus vix visibilibus magis impressis quam prominulis . . . . . 6d. *S. exigua* var. *tenerrima*

Fructus (5–)7–9 mm. longi; folia interdum paullo latiora, venis lateralibus pl.m. distincte prominulis 8b. *S. longifolia* var. *pedicellata*

Folia etiam adulta pl.m. sericea, utrinque (praecipue subtus) canescentia, venis lateralibus haud vel vix prominulis, ramulorum fertilium pl.m. lineari-lanceolata, integerrima vel satis distincte remote breviter denticulata; amenta fructifera pl.m. densiflora, fructibus pedicello glandulam saepe duplo superante instructis 6b. *S. exigua* var. *stenophylla*

Ovaria semper distincte sed interdum tantum pro parte sericeo-villosa vel sericea, fructus interdum fere vel omnino glabrescentes, subsessiles vel pedicello quam glandula pl.m. breviore suffulti, rarius distincte sessiles.

Folia ramulorum fertilium pl.m. integerrima vel tantum ad apicem parce et saepe indistincte denticulata, utrinque pl.m. canescentia, satis dense sericea vel etiam adulta non distincte glabrescentia et viridia venis etiam primariis vix vel paullo prominulis; fructus satis breviter conico-rostrati, amenta fructifera densa.

Folia etiam semiadulta utrinque (praesertim subtus) dense argenteo-sericeo-villosula, ramulorum fertilium saepe satis lanceolata, vulgo ad 8–10 mm. lata; ovaria juvenilia dense et longe sericea vel sericeo-villosula; fructus ellipsoideo-conici, 5–6.5 mm. longi (confer etiam 6c. *S. exiguam* var. *luteo-sericeam*) 5. *S. argophylla*

Folia minus dense, saepe tenuiter breviter adpresse sericea, ramulorum fertilium linearia vel lineari-

lanceolata, vulgo vix ultra 8 mm. lata vel ovaria angustiora apice magis capitata (incrassata) et fructus magis elongati .....6. *S. exigua*

Folia ramulorum fertilium pl.m. distincte denticulata, vulgo cito utrinque viridescentia et glabrescentia, adulta intense laete viridia et glabra (vel in var. *Wheeleri* utrinque pl.m. sericea), nervis etiam secundariis utrinque pl.m. prominulis; fructus magis elongati et rostrati; amenta fructifera pl.m. laxiflora (si amenta sunt valde densiflora et ovaria parce vel partim pilosa conf. etiam *S. melanopsidem* var. *Bolanderianam*)...8. *S. longifolia*

Glandula florum masculorum tantum una ventralis (rarius dorsalis minima adest); amenta feminea saltem novella ovariis dense albo-sericeo-villosis subsessilibus pl.m. micantia; glandula satis lata; folia ramulorum fertilium pl.m. linearia, 4–8 cm. longa et 1–5 mm. lata, ut in *S. longifolia* dentata et nervata.....8c. *S. longifolia* var. *angustissima*

Enumeratio specierum

1. S. TAXIFOLIA Kunth in Humb. and Bonpl., Nov. Gen. Pl. **2**:18. 1817; Sargent, Silva N. Am. **9**:129. *pl. 476*. 1896; Man. Trees N. Am. 175. *fig. 147*. 1905; Sudworth, Nomencl. Arb. Fl. U.S. 123. 1897, pro parte; Britton and Shafer, N. Am. Trees 202. *fig. 164*. 1908; for further literature and synonymy see SCHNEIDER in BOT. GAZ. **65**:23. 1918.—At present I have nothing to add to what I have already stated (*l.c.*) with regard to this species and its var. *microphylla* (Schl. and Cham.) Schn. There are several forms which look rather similar to *S. taxifolia*, but differ in the shape of the anthers and some other respects. I shall discuss them under *S. exigua*.

2. S. SESSILIFOLIA Nutt. N. Am. Sylva **1**:68. 1843,[2] reprint 1852; Anders. in Öfv. K. Vet.-Akad. Förh. **15**:116. 1858; in Proc. Amer. Acad. **4**:56 (Sal. Bor.-Am. 10). 1858; in Walp., Ann. Bot. **5**:746. 1858, incl. var. *villosa;* in K. Sv. Vet.-Akad. Handl. **6**:55. *pl. 4. fig. 36* (Monogr. Salic.). 1867; in DC. Prodr. **16**[2]:214. 1868;

[2] NUTTALL'S vol. 1 was issued in 2 parts; part 1 in 1842, containing pp. 1–54; while part 2, pp. 57–136, including the Salices, appeared in 1843.

Bebb in Watson, Bot. Calif. **2**:85. 1879,[3] pro parte et exclud. synon.; Sargent, Rep. For. N. Am. 10th Census U.S. **9**:168. 1884, pro parte et excl. var.; Silva N. Am. **9**:127. 1896, pro parte; Sudworth in Bull. U.S. Dept. Agric. Div. For. **14**:122 (Nomencl. Arb. Fl.). 1897, pro parte; For. Trees Pac. Slope 223. 1908, pro parte; Eastwood, Handb. Trees Calif. 37. 1905, pro parte; Britton and Shafer, N. Am. Trees 196. 1908, pro parte minima; Howell, Fl. Northw. Am. **1**:618. 1902; Piper in Contr. U.S. Nat. Herb. **11**: 213 (Fl. State Wash.). 1906; Ball in Bot. Gaz. **60**:49. *fig.* **2**. 1915; in Piper and Beattie, Fl. Northw. Coast 115. 1915; Henry, Fl. S. Br. Col. 96. 1915; Rydberg, Fl. Rocky Mts. 192. 1917, pro parte. —*S. sessilifolia* var. *villosa* And. in K. Sv. l.c. 56 et Prodr. l.c. 214.— *S. macrostachya* Nutt., N. Am. Sylva **1**:72. 1843; Howell, Fl. l.c. 619, pro parte; Rowlee in Bull. Torr. Bot. Club **27**:250. 1900, pro parte et excl. var.; Rydberg, Fl. l.c. 192.—*S. macrostachya* var. *Cusickii* Rowlee, in Bull. l.c. *pl. 9, fig. 5*, sine descr.—*S. longifolia* var. *sessilifolia* Jones, Willow Fam. 24. 1908.

Type locality.—Oregon, "on the rocky borders of the Oregon [Columbia] at the confluence of the Wahlamet" [Willamette]. Range: from western Oregon, Douglas County, along the Umpqua and Willamette River to the Columbia and Lewis rivers in Washington, thence again in northern Washington, Whatcom County, and southwestern British Columbia.

S. sessilifolia was the only one of Nuttall's species which has been correctly interpreted by Andersson, who cites for the type *Lyall's* specimens from the Sumass Prairie, of which the male is no. 78 and the female no. 31 in Herb. K.[4] They were collected in 1858 "near the 49th parallel of lat." In the herbarium Andersson first had named the specimen *S. Grayi*, but this name has never been published. For his var. *villosa* the type was collected by *Lobb* in 1852 in Oregon, bearing the no. 218 in Herb. K. *Lobb's* and *Lyall's*

[3] Bebb's treatment of the Californian Salices in Watson's *Flora* was published separately in 1879.

[4] Besides the abbreviations mentioned in Bot. Gaz. **65**:9 and **66**:121, the following will be used: Cal., Herbarium of the California Academy of Science; K., Kew Herbarium; Jeps., Herbarium of Professor W. L. Jepson, Berkeley, Cal.; N.E., Herbarium of the New England Botanical Club; P., Herbarium of the Academy of Science at Philadelphia, Pa.; Reno, Herbarium of the Nevada Agric. Exper. Station, Reno, Nev.; St., Herbarium of the Leland Stanford University.

plants are exactly alike. NUTTALL himself gave an excellent description, and it is rather astonishing that the species could have ever been misunderstood. He described another species, however, *S. macrostachya*, "from the banks of the Oregon" [Columbia], of which there is a sterile(!) cotype in Herb. P. and a branchlet with an old fruiting ament in Herb. G. According to some remnants the style and the stigmas are exactly as in *S. sessilifolia*, and there is no difference in the shape and pubescence of the leaves. Judging by NUTTALL'S statement "amentis longissimis praecocibus," he had before him a very early flowering state, and the fragment in G. shows an old, almost sessile, long, fruiting ament which naturally looks very different from the normal late flowering form with the aments at the top of rather long leafy branchlets. The sheet in P. also contains a female branch of which I do not know the origin, because it is only partly represented in the photograph. It seems to me that this branchlet belongs to the true *S. argophylla* Nutt., which has a similar foliage and pubescence but shorter stigmas, looking more or less intermediate between *S. sessilifolia* and *S. exigua*. I shall deal with it later. It has been mostly taken hitherto for *S. macrostachya*. BALL (1915) also referred specimens from California to *S. sessilifolia*, but those forms I take for var. *Hindsiana*. Sterile specimens collected by *J. G. Jack* in Oregon, Josephine County, Grant's Pass, August 23, 1904, and at the same locality and time by *A. Rehder*, seem to me to belong rather to *S. argophylla* than to *S. sessilifolia*. In British Columbia, Westminster County, New Westminster, banks of Fraser River, *J. K. Henry* collected good material on June 24, 1912, and May 9 and September 25, 1914 (m., fr., st.; Cal.). The largest leaves I have seen measure up to 9:2 cm.

2b. S. SESSILIFOLIA var. HINDSIANA And. in Öfv. K. Vet.-Akad. Förh. **15**:117. 1858; in Proc. Am. Acad. **4**:56 (Sal. Bor.-Am. 11). 1858; in Walp., Rep. Bot. **5**:746. 1858; Bebb in Watson, Bot. Calif. **2**:85. 1879; Sargent, Rep. For. N. Am. 10th Census U.S. **9**:169. 1884, excl. synon. var. *tenuifolia;* Eastwood, Handb. Trees Calif. 38. 1905.—*S. Hindsiana* Benth. Pl. Hartw. 335. 1857; Torrey in Pacif. R.R. Rep. 4^5:138. 1857; Newberry in Pacif.

R.R. Rep. 6³:89. 1857; And. in K. Vet.-Acad. Handl. 6:56 (Mon. Salic.). 1867, excl. *pl. 4, fig. 37* et var.—*S. longifolia* var. *argyrophylla* f. *angustissima* And. l.c. 55; in DC. Prodr. 16²: 214. 1868. sec. specim. Fremontii.—*S. longifolia* Greene, Man. Bot. S. Fran. Bay 299. 1894, pro parte max.—*S. sessilifolia* Sarg., Silva N. Am. 9:127. 1896, pro parte; Jepson, Fl. Calif. 339. 1909, pro parte max.; in Mem. Univ. Calif. 2:178 (Silva Calif.). 1910, prop arte max.; Ball in BOT. GAZ. 60:51. 1915, pro parte.

TYPE LOCALITY.—California, "ad ripas fluvii Sacramento." Range: central California to southwestern Oregon.

Of *S. Hindsiana* I have seen a photograph of the type (K.) and cotypes (G., N.) collected by *Hartweg*, which are all perfectly identical. It is closely related to typical *S. sessilifolia*, from which it differs chiefly by its more linear or narrowly lanceolate and almost always entire leaves, which are more or less distinctly petioled, and by its usually smaller and thinner aments. If it were not for some specimens which seem to combine var. *Hindsiana* with the northern *S. sessilifolia*, and others that I can hardly distinguish from the southern var. *leucodendroides* (for instance a vigorous sterile specimen from Yolo County, mouth of Buckeye Creek, lg. *R. Stinchfield*, no. 334; St.), I should take it for a distinct species. A closer study of those forms in the field is certainly needed.

There seems to occur a form with almost glabrate ovaries, judging by a specimen collected by *R. S. Ferris* in Colusa County, Sycamore Slough, April 17, 1917 (no. 619, m., f.; St.). It is otherwise rather typical var. *Hindsiana* and needs further study.

The range of this variety extends to Jackson County in southern Oregon (*Walpole*, no. 255; *Applegate*, nos. 624 and 2198) in the north, and to Monterey[5] and Kern counties in California in the south, but the southern forms like *Piper's* no. 6406 from Bakersfield come very near var. *leucodendroides*.

2c. S. SESSILIFOLIA var. LEUCODENDROIDES Schneider in BOT. GAZ. 65:26. 1918.—*S. macrostachya leucodendroides* Rowlee in Bull.

[5] From this county is *Brewer's* no. 544, which came from the Nacimiento or Nacimento River or Creek, not "Narsismente" or "Nasismento" River, as the name is spelled by ROWLEE and BALL according to the label in C. It is a male specimen with leaves much like var. *leucodendroides*, to which it may belong after all.

Torr. Bot. Club **27**:250. *pl. 9. fig. 6.* 1900; Abrams, Fl. Los Angeles suppl. ed. 102. 1911.—*S. integrifolia* var. *leucodendroides* Rowl., l.c. sphalmate in textu.—*S. argophylla* Rowl., l.c., pro parte.—*S. exigua* var. *virens* Rowl., l.c. 256, pro parte.—*S. sessilifolia* Eastwood, Handb. Trees Calif. 37. 1905, pro parte; Britton and Shafer, N. Am. Trees 196. 1908, pro parte; Jepson in Mem. Univ. Calif. **2**:178 (Silva Calif.). 1910, pro parte.—*S. macrostachya* Abrams, Fl. l.c. 101, non Nutt.—ROWLEE cites 3 specimens from southern California under his variety, namely *Parish's* nos. 2134, 2040, and 640. The last number is quoted by him also under his *S. argophylla*. It belongs to var. *leucodendroides*. No. 2134 represents an early flowering state of the male plant with small leaves and short peduncles of the catkins which measure up to 2:0.9 cm. The bracts are almost glabrate and often somewhat denticulate at apex, a fact we may also observe in other forms of *S. sessilifolia*. No. 2040, in my opinion, can be regarded as the typical var. *leucodendroides*, which seems to differ from var. *Hindsiana* chiefly in its comparatively longer and broader, very often distinctly denticulate leaves (with fine distant teeth), measuring usually from 7:1.2 to 13:1.8–2 cm. (in var. *Hindsiana* the corresponding entire leaves are about 3–10 cm. long and 3–10 mm. wide, while in the typical *sessilifolia* they measure from 5:0.8–1 to 8:3 cm., being distinctly denticulate with fine linear teeth), and by its stigmas, which usually are almost sessile and somewhat shorter and broader than in var. *typica* or var. *Hindsiana*. Some plants look almost like hybrids with *S. Parishiana* or the form of *S. exigua* from southern California. I can but repeat that a proper understanding of all these forms can only be gained by a careful study of them in the field. See also my remarks under *S. Parishiana* and *S. argophylla*.

I give an enumeration of the specimens I am inclined to refer to var. *leucodendroides*, and I should be glad to receive some information by collectors who visit these localities as to the different forms of willows growing together there.

SPECIMENS EXAMINED.—San Diego County: Santa Ysabel Creek, May 1893, *R. D. Alderson* (no. 700, f.; Cor.; ovariis parce sericeis; cited by ROWLEE under *S. exigua virens*); Mountain Spring, May 10, 1894, *E. A. Mearns* (no.

3040, m.; W.).—Riverside County: Santa Ana River, N.W. of Corona, very common, 150 m., May 26, 1918, *I. M. Johnston* (no. 1994, m., f.; A.); same place, 180 m., very common along river banks, June 9, 1917, *Crawford* and *Johnston* (no. 1244, m.; A.; St.); Temescal Canyon, along a dry wash, 400 m., May 30, 1918, *I. M. Johnston* (no. 2017, fr.; A.); same river, near Riverside, May 1888, *S. B. Parish* (no. 2040, f., type; C., Cor.); San Jacinto, along San Jacinto River, March 31, 1896, *A. J. McClatchie* (m., f.; N.; early flowering form, somewhat uncertain); eastern base of San Jacinto Mts., along the borders of the Colorado Desert, June 1901, *H. M. Hall* (no. 2105, m., f.; M.; ovariis laxe sericeis, stigmatibus mediocribus); San Jacinto River Canyon, gravelly ground along the river, common, May 12, 1918, *Durand* and *Street* (no. 23, f.; A.).—Orange County: Santa Ana River, June 1880, *S. B. Parish* (m.; A., M.; "12 ft. high").—Los Angeles County: Los Angeles, 1879, *J. C. Nevin* (m.; G.; fragment); San Gabriel River at El Monte, common along river, 90 m., May 13, 1917, *I. M. Johnston* (no. 1242, m., f.); same place, July 7, 1887, *Tracy* and *Evans* (no. 383, m.; N.); San Gabriel Mts., San Antonio Canyon, 1450 m., July 9, 1918, *F. G. Peirson* (no. 14, m.; Jeps.); canyon near San Rafael, March 31, 1888, *H. E. Hasse* (no. 3801, f.; N.; var. *Hindsianae* valde similis); sandy flat along the Los Angeles River, May 30, 1888, *H. E. Hasse* (no. 4092, m., f.; N.; stigmata iis *S. exiguae* satis similia); Los Angeles River bottom, near Los Angeles, September 9, 1917, *F. Grimmel* (fr.; St.).—San Bernardino County: San Bernardino Valley, dry sandy banks of Lyth Creek, in a large thicket, April 4, 1891, *S. B. Parish* (no. 2134, m. syntype; Cor. and C., both named *S. macrostachya* by ROWLEE; "about 4 ft. high"); Lyth Creek Wash, damp land, alt. circ. 300 m., May 2, 1917, *S. B. Parish* (no. 11134, f., fr.; A.; fructibus satis glabris); vicinity of San Bernardino, alt. 300–750 m., April 8, 1899, *S. B. Parish* (no. 4591, m.; St.; 4592, f., fr.; St.; the last number represents a small-leaved form much resembling *S. taxifolia* as well as var. *Hindsiana;* needs further observation); April 13, 1903, *S. B. Parish* (no. 5197, f.; St.; same small-leaved form); May 15, 1901, *S. B. Parish* (nos. 4786, m., 4787, f., fr.; N., St.; structura florum paullo ad *S. exiguam* vergens); March 1881, *S. B.* and *W. F. Parish* (no. 640, m., fr.; A.; var. *Hindsianae* satis similis, sed stigmatibus subbrevioribus, in C. magis typica); February 20, 1881, *W. G. Wright* (nos. 10, 11, m., 12, f.; C.; "small bush 6–10 ft."); March 1 and 14, 1881, *W. G. Wright* (nos. 6, m., 7, f.; C.; early flowering specimens with short aments which look rather different); Colton, April 28, 1882, *M. E. Jones* (m., fr.; A.); Waterman Canyon, August 1900, *Shaw* and *Illingworth* (no. 4, m.; St.; amentis brevibus, antheris parvis, sed foliis normalibus); Keenbrook, Kajon Pass, May 30, 1901, *S. B. Parish* (no. 4930, f., m.; St.; very much like *S. exigua*, but the female flowers more like those of var. *leucodendroides*); same Pass, July 6, 1908, *LeRoy Abrams* and *L. E. McGregor* (no. 694, f.; St.); Cucamonga Canyon, small colony on bed of a small side canyon, alt. 900 m., May 27, 1917, *I. M. Johnston* (no.

1241,[6] m.; St.).—Ventura County: Ventura, along beach, April 17, 1916, *A. Eastwood* (no. 5034, m., 5035, f.; Cal.).—Santa Barbara County: Santa Ynez River, alt. 600 m., May 1894, *C. Franceschi* (m.; A.; quasi ad var. *Hindsianam* transiens).—Tulare County: shores of Kern River, Peppermint Valley, alt. 1440 m., July 16, 1895, *W. R. Dudley* (no. 779, m.; St.); gravelly bars of Kaweah River at Three Rivers, July 20, 1900, *W. R. Dudley* (no. 2703, st.; St.); Three Rivers, near Brittons, June 15, 1902, *W. R. Dudley* (m., fr.; St.; all these forms of Tulare County come near var. *Hindsiana;* the fruiting aments of the last specimen measure up to 6:1 cm.). See also *Brewer's* no. 544 mentioned in the preceding note.

Specimens from Kern County, Bakersfield, September 28, 1910, *E. M. McGregor* (no. 13, m.; St.), look much like *S. exigua* and need further observation. There is a specimen from Santa Barbara County, Ojai, Cliff Glen, March 15, f., April 3, 1896, m., *F. W. Hubby* (no. 56; Cor.), of which the leaves much resemble *S. taxifolia*, but those of the more vigorous shoots seem to become larger. The female flowers have 2 glands, and the stigmas are rather short but agree with those of some forms I have referred to var. *leucodendroides*. I am not quite sure about this specimen, but I strongly suspect that it is a form of var. *leucodendroides* grown in a very arid position. It is similar to *Parish's* nos. 4591, 4592 already mentioned.

3. S. FLUVIATILIS Nuttall, N. Am. Sylva 1:73. 1843; Ball in BOT. GAZ. 60:52. *fig. 3.* 1915; in Piper and Beattie, Fl. Northwest Coast 114. 1915.—*S. sessilifolia* Sargent, Silva N. Am. 9:127. *pl. 475.* 1896, pro parte, non Nuttall; Rowlee in Bull. Torr. Bot. Club. 27:250. *pl. 9. fig. 8.* 1900; Howell, Fl. Northwest. Am. 1:618. 1902, pro parte; Sudworth, For. Trees Pacif. 223. *figs. 91, 92.* 1908, pro parte; Rydberg, Fl. Rocky Mts. 192. 1917, pro parte.—NUTTALL says: "This species lines the immediate border of the Oregon [Columbia] a little below its confluence with the Wahlamet"

[6] No. 1243 of the same collector from Red Hill, near Upland, April 28, 1917, apparently represents the female form of the same willow. Mr. JOHNSTON kindly sent me the following note regarding this number: "1243 from Cucamonga Canyon. Small colonies of this willow occur in scattered localities in the lower canyons of the San Antonio Mountains; although common in the valley it is uncommon in the mountains. 1243 came from one of these isolated colonies, and from absolute knowledge I know that no other colony of this or any other LONGIFOLIAE occurs within 3 miles. The associated *Salix* spp. were *S. laevigata* and *S. lasiolepis*. Nothing like *S. exigua* occurs for miles. This is by no possibility a hybrid." Judging by the stigmas this form is more closely related to *S. exigua* than to *S. sessilifolia*. The forms of this part of S. California need a special study, and it is almost impossible to express a definite opinion on them as long as *S. Parishiana* and *S. exigua* and its varieties are not yet properly understood.

[Willamette], and "we met this species likewise on the bank of the Lewis River of the Shoshonee." The first locality has been visited by BALL, and I follow him in his interpretation of this species. Unfortunately no type specimen exists, and from NUTTALL'S statement that "the germ is smooth, with 4 sessile stigmas" I believe that he had partly *S. melanopsis* before him from the second locality quoted, which is on the Snake River in western Idaho. At present the true *S. fluviatilis* is only known from "the lower part of the Willamette River and adjacent Columbia River" in Oregon, Multnomah County, ranging eastward to Wasco County, The Dalles, where *Ball* collected it on June 24, 1915 (nos. 1997, m., 1998, 1999, f., 2000, androgyn., 2005, fr., 2007, m., 2015, fr.; C., G.). It has also been found on the opposite bank of the Columbia, in Klickitat County, Wash., by *Suksdorf*, April 23, May 31, 1881 (no. 6, f., m.; C. [7876]). Other specimens of *Ball's* (nos. 1857, 1858, 1859; fr. adult.; G.) from northeastern Utah, Cache County, Logan Canyon, above Logan, in my opinion are somewhat uncertain. They suggest certain forms of *S. melanopsis* var. *Bolanderiana*, and indeed *S. fluviatilis* seems in some respects to be quasi intermediate between *S. sessilifolia* and *S. melanopsis*. BALL himself says: "The species is quite different from the true *sessilifolia*. It is closely related to *S. melanopsis* Nutt." But he also states: "The style and stigmas indeed are very similar to those of true *S. sessilifolia*." In fact, specimens collected by BALL on the shores of the Umpqua River, near Roseburg, Oregon (no. 1961, 1962, f., fr.; G.), and distributed by him as "?*S. Bolanderiana* (×*sessilifolia*)," are somewhat similar to *S. fluviatilis*, which, however, seems to be a good species of a very local distribution, quite different in the structure of the male flowers from that of the *melanopsis* group.

4. S. PARISHIANA Rowlee in Bull. Torr. Bot. Club **27**:249. *pl. 9. fig. 3.* 1900; Abrams, Fl. Los Angeles suppl. ed. 101. 1911.—*S. sessilifolia* Jepson, Fl. Calif. 339. 1909, pro parte, non Nutt.; in Mem. Univ. Calif. **2**:178 (Silva Calif.) 1910, pro parte.—*S. longifolia* var. *argyrophylla* Jeps. in Mem. l.c. pro parte.—*S. argophylla* Abrams, l.c. 102, pro parte.—This is a peculiar and rather obscure species of which ROWLEE has given a somewhat unsatisfactory description.

As type is cited *Hobby's* (recte *Frank Hubby*[7]) nos. 54, 55 from Matilija Canyon, Ventura County (not in San Bernardino County or, as is written on the label of the type no. 54 before me, Santa Barbara Co.). Besides this there is given on the label for the female specimen "Cliff Glen," and for the male "Ojai Springs," localities near Matilija. The flowers are young, and the ovaries are not "densely villous" but, at least partly in no. 55, glabrescent toward the apex and base, and rather silky pubescent. The specimens could easily be taken for *S. exigua* were it not for the fact that the lobes of the stigmas are narrower, about 3 times as long as thick, and the styles distinct but short. ROWLEE also cites a specimen collected by *Coville* and *Funston* (no. 263) at Spring Valley, Inyo County, but this is sterile. Only in Herb. W. I have found a few fruits attached to it which look much like those of *S. exigua*. I find it difficult to express a definite opinion on *S. Parishiana*, but I wish to enumerate the following specimens which may represent the same form. It looks intermediate between *S. exigua* (of southern California) and *S. sessilifolia* var. *leucodendroides*, and similar forms seem to occur in the region where var. *Hindsiana* reaches the southern limit of its range. The question whether we have to do with forms of hybrid origin or with a distinct species can only be solved by careful observation in the field. See also the indications given in the key.

SPECIMENS EXAMINED.—California: Venturia County: Matilija Canyon (see the remarks given in the preceding text), April 3, 1896, *F. W. Hubby* (no. 54, m. and f. types; Cor.), April 19, 1896, *F. W. Hubby* (no. 55, fr.; Cor.); Mt. Pinos Region, Goodenough Meadow, June 28, 1896, *W. R. Dudley* and *A. F. Lamb* (no. 4717, fr.; St.; fructibus parvis vix 5 mm. longis probabiliter nondum perfecte maturis); Sespe Creek, near Ten Sycamore Flat, alt. 600–750 m., June 9, 1908, *Abrams* and *McGregor* (no. 169; G., St.); Mt. Pinos Region, below Snedden's, Lockwood Creek, June 23, 1896, *Dudley* and *Lamb* (no. 4632, st.; St.; vel *exigua*). Los Angeles County: Burbank, 1904, *J. C. Nevin* (m., fr.; St.; very near *S. exigua*); Inglewood, April 12, 1901, *LeRoy Abrams* (no. 1493, f.; St.; glandulis 2, forma incerta); Florence, old bed of the Los Angeles River, April 13, 1903, *L. Abrams* (no. 3255, m., f.; M., St.; in floribus femineis interdum glandula dorsalis adest); same county?, Leakside, *J. B. Grant* (no. 6960, f.; St.; "shrub 8 ft. high"); San Antonio

[7] For correct statements regarding this name and the following localities I am much indebted to Mr. S. B. PARISH.

Mts., Prairie, fork of San Gabriel River, moist ground in a small open flat, alt. 1700 m., August 23, 1917, *I. M. Johnston* (no. 1685 m.; St.); San Bernardino County: San Bernardino, May 15, 1913, alt. 400 m., *W. L. Jepson* (no. 5591 m., fr.; A.). Orange County: Santa Ana, spring 1902, *H. D. Geis* (no. 653 vel 553; f., fr.; St.). San Diego County: Oneonta, April 24, 1904, *H. P. Chandler* (no. 5116, f., fr., m.; N.; porro observanda); near Tia Juana, June 1895, *S. G. Stokes* (f.; St.; stigmata pl.m. sessilia, forma porro observanda); same place, April 24, 1913, *A. Eastwood* (no. 2926, m.; A.); Tia Juana River, August 1902, *A. C. Herre* (fr.; St.; ut no. 4632).—Northern Lower California: Causito(?), May 29, 1883, *C. R. Orcutt* (no. 1180, fr.; M.; ut praecedens, sed amentis duplo brevioribus, ovariis pedicello quam glandula pl.m. sublongiore instructis). Kern County: along the Santa Fe Railroad, in low moist ground about 2 miles west of Bakersfield, April 6, 1905, *A. A. Heller* (no. 7591, m., f.; A., C., M., St.; looks somewhat like *S. exigua*×var. *Hindsiana;* "shrub 6 or 8 ft. high"). Inyo County: on the old Mitchell Range, resting Spring Valley, alt. 525 m., February 6, 1891, *F. V. Coville* and *F. Funston* (no. 263, st.; W.; see preceding remarks). Tulare County: Tule River above Porterville, March 27, 1897, *W. R. Dudley* (no. 3578, f.; St.; pubescentia foliorum valde juvenilium fere ut in var. *Hindsiana*, sed ovaria parce pilosa iis *S. Parishianae* simillima).

5. S. ARGOPHYLLA Nutt. N. Am. Sylva **1**:71. *pl. 20.* 1843; Rowlee in Bull. Torr. Bot. Club **27**:252. 1900, pro parte; Howell, Fl. Northw. Am. **2**:618. 1902, pro parte; Piper and Beattie, Fl. Palouse Reg. Wash. 53. 1901; Piper in Contr. U.S. Nat. Herb., **6**:213 (Fl. Wash.). 1906, pro parte.—*S. macrostachya* Piper, l.c. 214 non Nutt.; Henry, Fl. S. Br. Col. 96. 1915.—*S. sessilifolia* Britt. and Shafer, N. Am. Trees 196. *fig. 156.* 1908, pro parte.—This species, in my opinion, has been misunderstood by almost every later author, owing probably to the inaccurate representation in NUTTALL'S plate. His Latin description runs:

Salix argophylla, foliis lineari-sublanceolatis acutis sessilibus integerrimis utrinque argenteo-sericeis, stipulis obsoletis, amentis serotinis diandris, capsulis villosis lanceolatis. Besides this he says: "This species becomes a small tree from 12 to 15 ft. in height, as silvery and white as the *Leucodendron argenteum*, the branches are brown, but the twigs are hoary with villous hairs. The leaves are very much crowded, soft, with whitish shining silky down, so abundant on either side as wholly to hide the veins, and nearly the midrib; they are also nearly without footstalks, entire on the margin, of a narrow linear outline and sharply acute, with a distinct bristly point, 1.5 to 2 inches long, and only about 3 lines wide. Stipules small and linear, seldom seen. The aments come out late with the leaves, and the flower branches produce 4–7 leaves. The male ament is small and narrow, with the scales lanceolate and villous, the female

aments are oblong, the capsules lanceolate and villous. . . . . We perceive no affinity that this species bears, except perhaps to the *S. angustifolia* of the borders of the Caspian, from which at the same time it is probably very distinct.

NUTTALL'S statements indicate that the main character of *S. argophylla* is the soft, villous, white pubescence which is also a characteristic of *S. sessilifolia* and *S. macrostachya*. He does not indicate the shape of the stigmas, owing probably to the fact that he collected only plants with mature capsules. The type locality is "one of the branches of the Oregon [Columbia], the river Boisée, toward its junction with the Shoshonee" [Snake River] in western Idaho, Canyon County. So far as I know there is no type in existence, but *Nelson* and *Macbride's* no. 1057 and *Macbride's* no. 228 from the same county seem identical with NUTTALL'S species. ANDERSSON mentioned it first in his monograph in 1867 as follows:

"*S. longifolia* ***argyrophylla:* (Nutt. Sylva Amer. p. 87 ?): foliis et capsulis tomento argenteo tomentoso-micantibus.—In regionibus meridionalibus, ut in Mexico, etc.," and he adds a forma "*angustissima:* foliis anguste linearibus." "Hab. in ripis in California (Fremont); Rocky Mountains (Nuttall)," giving as a synonym "*S. brachycarpa* Nutt. Amer. Sylva p. 85 ?."

In the Prodromus (1868) ANDERSSON cites under his *S. longifolia*, *argyrophylla Berlandier's* no. 2371 (recte 2341) and *Wright's* no. 1873, and adds a forma *opaca*. He certainly misunderstood NUTTALL'S species entirely, and owing to the changed spelling of the name we may regard his var. *argyrophylla* as quite a new form which has nothing at all to do with *S. argophylla*. For a further explanation of ANDERSSON'S plant see under *S. longifolia* var. *angustissima*. *S. longifolia argyrophylla* of BEBB and other authors as well as *S. fluviatilis argyrophylla* Sargent are names applied to forms of very different origin, and may sometimes include the true *S. argophylla*, but mostly seem to refer to *S. longifolia* var. *Wheeleri*. ROWLEE (1900) mixed with it *S. Hindsiana* Benth. and also forms which belong to *S. exigua* and *S. sessilifolia leucodendroides*. PIPER (1906) and BALL (in different herbaria) referred the forms I take for *S. argophylla* mostly to *S. macrostachya*, but NUTTALL'S type of this species belongs to *S. sessilifolia*, as previously explained.

Male or sterile specimens of *S. argophylla* are not always easily separated from *S. sessilifolia*, as for instance those collected by

Jack and also by *Rehder* on Grant's Pass, Oregon. The female plants show almost the same stigmas as in *S. exigua*, and *S. argophylla* looks often quite intermediate between this species and *S. sessilifolia*.

So far as can be judged at present by the specimens enumerated, its range seems to extend from Bonneville County in eastern Idaho, along Snake River to Canyon, Washington, and Nez Perces counties, and into adjacent Washington (Walla Walla, Whitman, and probably also Franklin and Lincoln counties) as far as western Klickitat County, while in Oregon the species occurs in Sherman and Wasco counties, the forms from Klamath and Josephine counties being rather uncertain. The male specimen from British Columbia cited later looks much like *S. sessilifolia*, but Professor PIPER, with whom I have had an opportunity to discuss the matter, believes it is better referred to *S. argophylla* for geographical reasons. Only a close study in the field, especially of the forms of southern Washington and northern Oregon in the region of the Columbia and its tributaries, can elucidate the relationship of *S. argophylla* with *S. sessilifolia* and the limits of their geographical distribution. At present I can hardly do more than to indicate what form has to be taken for NUTTALL'S *S. argophylla*, and how it seems to be related to and connected with either *S. sessilifolia* or *S. exigua*. It would be rather misleading to make too decisive statements as long as one's information is merely based on herbarium material.

SPECIMENS EXAMINED.—Idaho: Bonneville County: Idaho Falls, among rocks, along river, July 4, 1901, *E. D. Merrill* and *E. N. Wilcox* (no. 803, m.; G.; "4–5 ft."); Canyon County: Falk's Store, slough and creek banks, alt. 660 m., July 11, 1911, *A. Nelson* and *Macbride* (no. 1057, fr.; G., M., St.); along the river, same alt., June 7, 1910, *J. F. Macbride* (no. 228, m.; G., M., St.); Caldwell, irrigation ditch, October 1, 1910, *C. R. Ball* (no. 1705, fr.; W.; "10 ft."); Washington County: Weiser, alt. 660 m., July 5, 1899, *M. E. Jones* (no. 6554, f., fr.; W.); ?Nez Perce County: Clear Water River, June 18, 1894, *L. F. Henderson* (f., fr., non m.; W.; same as no. 2878 in C.; forma satis ad *S. exiguam* spectans).—Washington: Walla Walla County: Waitsburg, June 24, 1897, *R. M. Horner* (R. 454, B. 451, m.; G., W.); Whitman County: Wawawai, July 9, 1901, *C. V. Piper* (no. 3592, m.); same place and collector, June 13, 1901 (no. 3595, m.); West Klickitat County: Columbia River, damp or wet places, May 31, July 1884, *W. N. Suksdorf* (m., f., fr.; C.,

M., St.); Franklin County: Pasco, June 1902, *H. P. Baker* (no. 70, m.; M.; vel ad *S. sessilifoliam* referenda); Lincoln County: Sprague, alt. 560 m., June 3, 1893, *J. H. Sandberg* and *J. B. Leiberg* (no. 134; W.; forma porro observanda, paullo ad *S. exiguam* spectans).—Oregon: Sherman County: Biggs, along stream, 1 mile south of Columbia River, August 1, 1914, *C. R. Ball* (no. 1848, fr.; W.); Wasco County: Tygh Valley, June 1881 (vel 1880), *T. J. Howell* (m. vel androgyn.; A., M.); Hood River County, Hood River, May 25, 1879, *J. T. J. Howell* (m. vel androgyn.; C.); Klamath County: along Sprague River above Yainax Valley, *F. V. Coville*, August 23, 1902 (no. 1312, st.; forma quamvis incerta); Josephine County: Grant's Pass, August 23, 1904, *J. G. Jack* (st., A.; forma incerta); same place and date, *A. Rehder* (st.; "large shrub"; ut praecedens); ? County: Cache Bar, between Cache and Gordon creeks on Snake River, alt. 380 m., June 19, 1897, *E. P. Sheldon* (no. 8325, m.); east Oregon, without exact locality, stream banks, May 9, June 7, September 1898, *W. C. Cusick* (no. 1860, m., f., fr.; M.; "a straight upright shrub"; forma foliis lanceolatis satis denticulatis).—British Columbia: Kootenay District, Cascade, near international boundary between Kettle and Columbia rivers, June 26, 1902, *J. M. Macoun* (no. 68128, O.; m.; G.).

6. S. EXIGUA Nutt. Sylva N. Am. **1**:75. 1843; Rowlee in Bull. Torr. Bot. Club **27**:255. *pl. 9, fig. 15*. 1900, pro parte; Piper and Beattie, Fl. Palouse Reg. Wash. 53. 1901; Howell, Fl. Northw. Am. **1**:618. 1902; Piper in Contr. U.S. Nat. Herb. **6**:213 (Fl. Wash.). 1906; Britton and Shafer, N. Am. Trees 195, *fig. 155*. 1908; Ball in Coult. and Nels., New Man. Rocky Mts. Bot. 131. 1909; Garrett, Spring Fl. Wasatch Reg. 10. 1901, pro parte; ed. 2. 16. 1912, pro parte; Rydberg, Fl. Rocky Mts. 192. 1917, pro parte.—*S. longifolia* var. β Hooker, Fl. Bor. Am. **2**:149. 1839, quoad specim. Tolmieana.—*S. longifolia* Wats., Cat. Pl. Nev. Utah, in King's Rep. **5**:324. 1871, quoad specim. no. 1094, non Muhl.; Bebb in Coult., Man. Rocky Mts. Bot. 335. 1885, pro parte; Jeps. in Mem. Univ. Calif. **2**:178 (Silva Calif.) 1910, pro parte.—*S. longifolia* var. *exigua* Bebb in Wats., Bot. Calif. **2**:85. 1879; Jones, Willow Fam. 24. 1908, pro parte.—*S. longifolia* var. *argyrophylla* Macoun Cat. Can. Pl. **1**:450. 1883, pro parte; Jeps. in Mem. l.c. pro parte.—*S. fluviatilis* var. *exigua* Sarg., Silva N. Am. **9**:124. 1896, pro parte; Sudworth in Bull. U.S. Dept. Agr. Div. For. **14**:122 (Nomencl. Arb. Fl.). 1897, pro parte max; For. Trees Pacif. Slope 223. 1908.—*S. longifolia* var. *argophylla* Jones, Willow Fam. 24. 1908, pro parte.—*S. argophylla* Henry, Fl. S. Br. Col. 96. 1915;

Rydbg., Fl. Rocky Mts. 188. 1917.—The type of this species was collected by *Nuttall* with his *fluviatilis*, probably "on the banks of the Lewis River of the Shoshonee" (Snake River in Idaho), because at the type locality of *S. fluviatilis* on the Columbia in the vicinity of Portland, Oregon, this species is apparently the only one of the LONGIFOLIAE according to BALL (BOT. GAZ. **60**:45, in note, 1915). *Nuttall* says: "This species is also a native of the territory of Oregon, and grew with the preceding, which it strongly resembles" (*S. fluviatilis*); he does not indicate the exact locality. I have a photograph of a so-called cotype of *S. exigua* from Herb. P. consisting of a sterile branchlet. The label originally bore the inscription "*S. longifolia*, Missouri and Arkansas." The name *longifolia* has been crossed out, and in a similar handwriting is written "*exigua* Nutt." Judging by the serration and nervation of the leaves there can be no doubt that the specimen belongs to *S. longifolia*. I do not know of a true type specimen of *S. exigua*, but there can hardly be any doubt as to the form NUTTALL had in mind. From his phrase "capsulis lanceolatis sessilibus, demum nudiusculis" I infer that the typical *S. exigua* is a form with, at least in the beginning, hairy ovaries, but ROWLEE and other authors ascribe to it glabrous capsules. BALL (1909) is right in stating that it is "variable in foliage characters and sometimes very difficult to distinguish" from *S. longifolia*. In spite of having seen an abundant and well collected material, I am still at a loss how to define certain forms and to draw a sharp line between *S. exigua* on the one hand and such species as *S. longifolia*, *S. argophylla*, *S. Parishiana*, and also *S. taxifolia typica* on the other. From *S. longifolia* and its forms it differs chiefly in the opaque color of the canescent leaf-surfaces, bearing a more or less dense appressed tomentum of short silky hairs (especially on the young leaves) of a silvery hue. The leaves are usually smoother with a hardly visible nervation, but in old leaves (for instance in those of the southern form) the veins are sometimes rather well marked; their margin is mostly entire, but a dentation similar to that of *S. longifolia* may be observed in the southern forms. The fruiting aments usually are denser and the capsules as a whole shorter. *S. argophylla* chiefly differs, as previously stated, by its more villous tomentum, while *S. Parishiana*,

which cannot be distinguished by its pubescence, may be recognized by the longer lobes of the stigmas and the more or less distinct style. Male specimens of these species sometimes prove difficult to distinguish. In *S. sessilifolia leucodendroides* the base of the leaves usually is more obtuse and suddenly contracted in the very short petiole, while in *S. exigua* as well as in *S. Parishiana* the leaves are mostly attenuated at the base, passing gradually into the somewhat longer petioles. *S. Parishiana* normally has linear leaves, while in *S. exigua* they are more linear-lanceolate, but all those characters have to be taken cum grano salis. There is a specimen before me from southern New Mexico, Dona Ana County, Mesilla, alt. 1150 m., June 19, 1897, *E. O. Wooton* (no. 39, m.; G., St., W.), of which the younger leaves are almost sessile, with a pubescence like those of var. *leucodendroides*, but are more linear; the older ones, which are more glabrescent and measure up to 12 by 0.5 cm., have a distinct petiole 2–3 mm. long. The pubescence and shape of the bracts seem to vary in the same manner in every species. Whether or not the shape and size of the anthers afford a useful character I cannot state. In those regions where the species meet each other hybrid forms are certain to occur.

The range of what I call the typical form of *S. exigua* extends from southern Idaho (from which the type probably came) westward to Oregon (where the western line seems to run from about Wasco County to Klamath County) and Washington (where it hardly reaches the eastern slopes of the Cascades), northward to British Columbia (where I did not see it from farther north and west than Clinton on the Fraser River) and southern Alberta (Medicine Hat), eastward to central Montana and western Wyoming (Yellowstone Park), and southward to southeastern Nevada and southern California. In California it seems to occur along the eastern border line from Modoc to Inyo County (Panamint Range), and in the south (Ventura to San Bernardino, Imperial, and San Diego counties). There are also forms very near to it in San Benito, Tulare, and Kern counties, which partly point toward *S. sessilifolia* var. *Hindsiana*. From the south I also have seen forms which come very near var. *leucodendroides* on the one hand and *S. Parishiana* on the other. As already stated, the limitation of these species is a very difficult task.

In Nevada and Utah a form is found in which the female flowers have a ventral and a dorsal gland. To this form belongs *S. nevadensis* Wats., the type of which came from Nevada, Ormsby County, near Carson City. It is certainly not a good species, but I am inclined to keep it as a variety until it is proved by further observation that the presence of a dorsal gland is a character of no taxonomic value, and that no other character can be detected by studying the plant in the field. In proposing the name S. EXIGUA var. **nevadensis,** nov. var. (*S. nevadensis* Watson in Am. Nat. **7**:302. 1873), I provisionally refer to it the following specimens, and wish to draw the attention of collectors to the localities mentioned. The type has glabrous ovaries, with a pedicel nearly as long as the ventral gland, while other forms with two glands have a more or less dense pubescence.

SPECIMENS EXAMINED.—Nevada: Ormsby County, at the base of the Washoe Mountains, near Carson City, alt. 1500 m., April 1868, *S. Watson* (no. 1093, f. type; G.); same region, 1865, *C. L. Anderson* (no. 196, m., fr.; G.; ovaria pilosa); Washoe County, Franktown Creek, May 18, 1907, *C. L. Brown* (no. 1677, f.; Reno); Glendale, alt. 1300 m., May 1, 1909, *P. B. Kennedy* (no. 1743, m.; G.); sloughs between Pyramid and Winnemucca lakes, alt. 1250 m., June 2, 1913, *P. B. Kennedy* (no. 1996, m., fr.; G.; forma quasi ad *S. sessilifoliam* var. *Hindsianam* accedens); Truckee River, alt. 1350 m., June 6, 1913, *P. B. Kennedy* (no. 2010, m., f.; G.); central Nevada, without exact locality, 1871, *Wheeler* (m.; syntype; G.).—California: Nevada County, along Coldstream, 3 miles above Truckee, July 17, 1913, *A. A. Heller* (no. 6953, fr.; forma aliquid incerta).—Utah: Washington County, St. George, alt. 600 m., April 9, 1880, *M. E. Jones* (no. 1644, m., f.; A., C.); without date, *E. Palmer* (no. 8, m., f.; M.); Redsand, alt. 900 m., April 24, 1894, *M. E. Jones* (no. 5117, m., f.; M.); Santa Clara, 1874, *C. C. Parry* (no. 8, m., f.; M.); Beaver County, Milford, along a stream, June 4, 1902, *L. N. Goodding* (no. 1018, fr.; W.); plains and mountains east of Milford, June 22, 1905, *P. A. Rydberg* and *E. C. Carlton* (no. 6318, fr.; G.); Salt Lake County, Salt Lake City, 1350 m., May 1869, *S. Watson* (no. 1091, fr.; G.); same place, May 12, 1880, *M. E. Jones* (no. 1710, m., f.; A., C.); Davis County, Lagoon, common, alt. 1500 m., July 7–8, 1901, *Pammel, Johnson, Buchanan,* and *Lummis* (fr. adult.; M.; probably var. *typica*).—Idaho: Bear Lake County, Montpelier, creek banks, May 20, 1910, *J. F. Macbride* (no. 207, f.; G.); Power County, north of Arbon, bridge over Bannock River, August 6, 1915, *C. R. Ball* (no. 2020, st.; G.; forma incerta).

There are also the following 2 specimens from southern California which resemble *S. exigua* and possess 2 glands in the female flowers: San Bernardino

County, Cushenberry[8] Spr[ing], Mojave Desert, June 2, 1901, *S. B. Parish* (no. 4931; N., St.), and Los Angeles County, Los Angeles, April 1901, *G. B. Grant* (no. 1156; M.; apparently the same as *Parish's* plant). Both need further observation.

In 1900 ROWLEE described a *S. exigua* var. *virens* (Bull. Torr. Bot. Club **27**:255), for the type of which a specimen collected by *Rothrock* in Arizona has to be taken. So far as can be discovered from the specimens cited by the author, I believe that ROWLEE mixed several forms of different affinity, belonging partly to *S. melanopsis Bolanderiana* (*Bolander*, no. 5031; *Kellogg* and *Harford*, no. 922; *W. G. Wright*, Kernville [not Kernerville]), and partly to *S. sessilifolia leucodendroides* (*Alderson* [not *Anderson*] no. 700). The type of *Rothrock*, which is sheet no. 6122 in C., represents a female specimen of which the flowers can hardly be distinguished from those of *S. exigua*. In the leaves it agrees well with a male specimen of *Orcutt's* (San Diego County, in the southwestern part of the Colorado Desert, Dos Cabesas, October 11, 1890, no. 2227; A., C.), which number is also cited by ROWLEE. Both may be taken for a rather glabrescent variety of *S. exigua*, but the leaves show under the lens a fine and thin silky pubescence and cannot be called "nearly glabrous," a character apparently taken by ROWLEE from the specimens of var. *Bolanderiana*. *Rothrock's* and *Orcutt's* specimens come very near the 2 specimens of *Parish* and *Grant* with 2 glands in the female flowers. Besides these there is *Parish's* no. 3194 (San Bernardino County, San Bernardino Mountains, Big Morongo, alt. 900 m., June 15, 1894; m.; M.) that hardly differs from *Orcutt's* plant, and also *LeRoy Abrams'* and *McGregor's* no. 406 (Los Angeles County, Liebre Mountains, Oakgrove Canyon and Elizabeth Lake, June 20–23, 1908; f., fr.; St.) seems to represent such a form the leaves of which become rather greenish at maturity, but the lower surface is rather glabrescent in *Rothrock's* specimens. This form somewhat simulates var. *Bolanderiana*, and I cannot express at present a definite opinion as to its real taxonomic value and true affinity.

[8] ROWLEE spells the name Cashewberry, but I read it as given, and S. B. PARISH writes in a letter to Professor C. S. SARGENT that this is the local way of spelling the name, while on the map of the Geological Survey it is spelled Cushenbury.

In BOT. GAZ. **65**:25. 1918 I have made *S. stenophylla* Rydbg. a variety of *S. exigua*, referring to it the eastern and southeastern forms of this species. RYDBERG's female type and male syntype came from southern Colorado, Huerfano County, Cuchara River, below La Veta (*Rydberg* and *Vreeland*, nos. 6393 f., 6392 m.; N.), and the ovaries are only partly glabrous, while most of the forms I take for var. *stenophylla* have wholly glabrous ovaries and fruits. The main character by which they differ from typical *S. exigua* is the longer pedicel, which in the fruit usually surpasses the gland in length. After all, even this character can scarcely be regarded as constant, and var. *stenophylla* is connected with the typical form by numerous intermediates. As a whole, however, the forms of *S. exigua* from Wyoming, Colorado, Arizona, New Mexico, Texas (Randall and El Paso counties), and probably also on the western border of Kansas, in northwestern Oklahoma, and in northern Mexico (northern Chihuahua), seem to present slight variations and may be called var. *stenophylla* until further studies in the field have led to a more proper understanding of the variability of this species. I suggested in BOT. GAZ. **65**:25. 1918 that *S. Hindsiana* var. *tenuifolia* And. (in K. Sv. Vet.-Akad. Handl. **6**:56. 1867) might be identical with var. *stenophylla*, in which case the name *tenuifolia* would have to be used. As type a specimen collected by *Burke* on the banks of the Snake River near Fort Hall in Idaho has to be taken. Judging by a photograph and fragments of the type preserved in Herb. K. I cannot decide whether the male specimen really belongs to what I call var. *stenophylla* or to the typical *S. exigua*. It comes from a region where both forms meet. The second specimen cited by ANDERSSON "Nova Mexico (*Schur*)" is unknown to me, and may probably be referable to var. *stenophylla*, which name I prefer to keep so long as the identity of the Snake River form remains uncertain. To var. *stenophylla* also partly belongs as a synonym *S. longifolia* * * * *opaca* And. (in K. Sv. Vet.-Akad. Handl. **6**:55. 1867) in so far as it refers to *Wright's* no. 1873, while *Berlandier's* no. 2341 represents *S. longifolia angustissima*.

In western Nebraska and northeastern Colorado another form of *S. exigua* has been found which somewhat reminds one of the f.

Wheeleri of *S. longifolia* (see following). RYDBERG described this form as *S. luteosericea* (in Britton, Man. 316. 1901) and kept the name in his Fl. Color. 94. 1906, while he makes it a synonym of his *S. exigua* in 1917 (Fl. Rocky Mts. 192), as BALL has already done in 1909. The type came from western Nebraska, Banner County. I think it best at present to keep this form separate under the name S. EXIGUA var. **luteosericea,** nov. var., and I provisionally refer the following specimens to it in the hope that collectors may pay attention to the localities mentioned and try to get a better understanding of this variety by studying it carefully in the field as to its association with typical *exigua* and with *S. longifolia*. I can hardly point out a good character by which to recognize this form, but its pubescence is a little more villose, and the aments are more loosely flowered than in typical *exigua* or var. *stenophylla*.

SPECIMENS EXAMINED.—Western Nebraska: Banner County, Lawrence Fork, July 8, 1891, *P. A. Rydberg* (no. 368 partim, f. type; N.); Kearney County, dry creek, June 13, 1891, *P. A. Rydberg* (no. 369, m. syntype; N.); Scotts Bluff County, Platte bottom, in Mitchell Valley, August 4, 1891, *P. A. Rydberg* (no. 368 partim, fr.; N.).—Colorado: Weld County, Greeley, July 23, 1896, *L. H. Pammel* (no. 200, fr., 201, m.; M.); Larimer County, without exact locality, plains, alt. 1500 m., June 26, 1895, *C. F. Baker* (*Patterson* no. 9842, m., f., rather typical, the male specimen almost identical with *exigua typica*); Fort Collins, near river, June 26, 1896, *L. H. Pammel* (no. 202, f.; M.); same locality, meadow near river, August 6, 1898 (Hb. Agr. Coll. Colo., no. 2343, fr.; C.); Morgan County, Fort Morgan, June 1896, *L. H. Pammel* (no. 204, st.; M.); Fremont County, Canyon City, banks of the Arkansas River, September 24, 1874, *G. Engelmann* (st., M.; vel var. *stenophylla*); Boulder County, August 1 [and 21 ?], 1884, July 20, 1885, *G. W. Letterman* (fr.; M.); Denver County, Denver, August 20, 1884, *G. W. Letterman* (fr.; M.).—S. Dakota: Butte County, Indian Creek, along flood plain, July 31, 1911, *S. S. Visher* (no. 2640, st.; C.; f. incerta); Bennett County, Little White River, valleys, August 15, 1911, *S. S. Visher* (no. 2274, st.; C.; rather uncertain, similar to *S. longifolia Wheeleri*).

There remains another form the proper interpretation of which raises many difficulties. It was described by HENDERSON as *S. longifolia tenerrima* from specimens collected by the author in Idaho, Elmore, and Canyon counties. At first sight it can hardly be distinguished from what I call *S. longifolia* var. *pedicellata* (see later), especially from such specimens as *Eastwood's* no. 465, but a

closer inspection shows that the leaves as a whole are narrower and the fruits shorter. In this respect it agrees more with *S. exigua*, of which it would represent an extremely glabrescent form. HELLER made it a species, and BALL evidently took the same view, as shown by his determinations of herbarium specimens before me, but RYDBERG (1917) quotes it as a synonym of his *S. linearifolia*, which is the same as var. *pedicellata* of *longifolia*. So far as I know the range of this peculiar form, it seems to be restricted to southwestern Idaho (region of Boise River), northwestern Wyoming (Yellowstone Park and northern Lincoln County), and adjacent southern Montana (Carbon and Big Horn counties). There is, to my present knowledge, no *S. longifolia* in this region, but it is within the range of *S. exigua*. I am therefore inclined to follow a suggestion of C. V. PIPER, with whom I have discussed this question, and to refer var. *tenerrima* as a variety to *S. exigua*.

S. EXIGUA var. **tenerrima,** nov. comb.—*S. longifolia* var. *tenerrima* Henderson in Bull. Torr. Bot. Club **27**:354. 1900.—*S. tenerrima* Heller, Cat. N. Am. Pl. ed. 2. 4. 1900.—*S. fluviatilis* var. *tenerrima* Howell, Fl. N.W. Am. 618. 1902.—*S. linearifolia* Rydbg., Fl. Colo. 94. 1906, ex parte; Fl. Rocky Mts. 192. 1917 ex parte.—A typo praecipue differt foliis angustioribus linearibus etiam maximis vix ultra 4 mm. latis juvenilibus ut rami novelli parce breviter sericeis cito glabris vel pilis parcis difficile recognoscentibus vestitis utrinque satis viridibus vix nervatis vulgo pl.m. distincte denticulatis dentibus brevibus subglandulosis saepe satis distantibus, ovariis subsessilibus glabris, bracteis oblanceolatis tantum versus basim pilosis, fructibus vulgo pedicello distincto glandulam duplo superante instructis conico-rostratis pedicello excluso ad 6 mm. longis.

SPECIMENS EXAMINED.—Idaho: Elmore County, shady rocky banks of mountain rills gone dry, July 12, 1895, *L. F. Henderson* (fr., type; G.); Canyon County, Payette River, sandy bottoms, August 1, 1897, *L. F. Henderson* (fr.; G.); Falk's Store, open sandy slopes, alt. 660 m., May 24, 1910, *J. F. Macbride* (no. 98 m., fr. juv.; G., M., St.; "loose clumps").—Wyoming: Yellowstone Park, Soda Butte Creek, July 14, 1899, in small clumps on the stony river bottom, *A.* and *E. Nelson* (no. 5866, fr.; G., St.); Lincoln County, Jackson's Hole, banks of Gros Ventre River, July 14, 1901, *S. D. Merrill* and *E. N. Wilcox* (no. 996, fr.; G., M.; "10 ft.").—Montana: Big Horn County, Crow Agency,

August 30, 1871, *Coulter* (no. 5, st.; C.; forma porro observanda); Carbon County, near Red Lodge, July 28, 1893, *J. N. Rose* (no. 50, fr. adult.; forma aliquid incerta); Gallatin County, Bozeman, Gallatin River, low ground, October 4, 1905, *J. W. Blankinship* (no. 465, st.; A.; forma incerta ad *S. longifoliam pedicellatam* spectans); Rosebud County, Forsyth, north of town, toward river, 1908, *C. R. Ball* (no. 1305, st.; G.; "6 ft. high"; forma porro observanda).—Utah: Cache County, Logan Canyon, above Logan, August 8, 1914, *C. R. Ball* (no. 1864, fr.; W.; forma glabra pro *S. exigua* determinata, porro observanda).

This variety needs further observation in the field, and some of the specimens cited are uncertain owing to the lack of fertile material. Some forms of *S. longifolia pedicellata* are extremely alike, but the leaves show a more or less prominent (often very fine) venation, while in the leaves of var. *tenerrima* the lateral veinlets are scarcely visible and finely impressed; the fruits of both are sometimes almost identical, and I am not yet sure of the true affinity of var. *tenerrima*. *G. J. Jack*, August 16, 1918, collected on the Laramie River, Laramie, Albany County, Wyoming (no. 1017), sterile specimens of a form of which I am not sure whether it is var. *tenerrima* or var. *pedicellata*, neither of which has hitherto been reported from southeastern Wyoming. Professor JACK says: "Slender, coarse, grasslike, 2–3 ft. high, covering wide sandy areas," and he told me that it is a very distinct low form. There are now living plants in the Arnold Arboretum which I hope will prove useful in determining its real affinity.

There is still one form which needs a few words. It was collected by *S. M. Tracy* and *F. S. Earle* in western Texas, Jeff Davis County, Limpia Canyon, April 24, 1902 (no. 210, fr.; C., G.; distributed as "*S. longifolia opaca* Ands."), and it seems to be identical with Mexican specimens mentioned by me in BOT. GAZ. **65**:23. 1918, under *S. taxifolia*. The habit and the leaves agree well with those of that species, but the fruits in no. 210 are much more like those of *S. exigua* with short sessile stigmas. It looks almost like a new species closely related to *S. exigua*, which seems to show a variability remarkable even among willows.

7. S. MELANOPSIS Nuttall, N. Am. Sylva 78. *pl. 21*. 1843; Rowlee in Bull. Torr. Bot. Club **27**:256. *pl. 9, fig. 16*. 1900, pro parte; Piper and Beattie, Fl. Palouse Reg. Wash. 53. 1901; Piper in Contr. U.S.N. Herb. **11**:213 (Fl. Wash.). 1906, pro parte; Ball

in Coult. and Nels., New Man. R. Mt. Bot. 131. 1909; in Piper and Beattie, Fl. Northw. Coast 114. 1915; Henry, Fl. S. Br. Col. 97. 1915; Rydberg, Fl. R. Mts. 192. 1917.—*S. longifolia* Bebb apud Coulter, Man. R. Mt. Bot. 335. 1885, pro parte, non Muhl.—*S. fluviatilis* Howell, Fl. Northw. Am. 1:618. 1902, pro parte, non Nutt.—This is a well marked species the type of which was found by NUTTALL "at our station called Fort Hall, in the plains of the Rocky Mountains, on alluvial lands of Lewis River of the Shoshonee." According to BALL (1909), this is old Fort Hall, near Pocatello, in Bannock County, eastern Idaho, south of the present Fort Hall,[9] near Blackfoot, in Bingham County. I have seen a photograph of a cotype preserved in Herb. P. BALL (1909) gives the range as follows: "Common in northeastern Oregon, eastern Washington, and British Columbia as far east as the Selkirks." I have not seen a specimen from the type region or other parts of southern Idaho, but only from northern Idaho, Montana (Teton County, Midvale, *L. M. Umbach*, no. 170), Alberta (Crow Nest Pass and Jasper), where it seems to reach its northern limit at about the 53d parallel, British Columbia (in the Chilliwack Valley and at Revelstoke), Washington (where I have seen it west of the Cascades only from King County, Snoqualmie), Oregon (where it was collected by *Ball* in 1915 as far west as the Umpqua River, Roseburg, Douglas County, and by *Applegate*, no. 2224, at Ashland, Jackson County), and northern and northeastern California (see below), where it seems to pass into var. *Bolanderiana*. According to BALL (BOT. GAZ. **60**:45, first note, 1915), *S. Bolanderiana* is associated with *S. sessilifolia* at Roseburg and also farther north "on the Willamette River at Corvallis," Benton County. What I have seen from Oregon I take for the true *S. melanopsis*, which ought to be looked for also in northern Utah and in western Wyoming.[10] Its

[9] This locality, however, is identical with that given for Fort Hall in Lippincott's Geogr. Dict., ed. of 1855; while on the map in the Century Atlas of 1911 old Fort Hall is indicated south of the 43d parallel just north of Pocatello. Judging by Rand McNally's map the whole region between the two places is called Fort Hall.

[10] There is a specimen from eastern Wyoming, Converse County, Rawhide Creek, south of Patrick, August 27, 1901, *H. P. Baker* (m.; M.), which looks like typical *S. melanopsis*. In Herb. C. I found a specimen from Colorado, Clear Creek County, damp places along Clear Creek, 1885, *H. N. Patterson* (fr. adult. [sheets 5523 and 107801]), which clearly resembles *S. melanopsis*. I am not sure whether the localities given are correct.

occurrence so far north in Alberta is interesting. In the north a form with more hairy, almost shining silky leaves seems to be not infrequent (see *J. Macoun's* specimen from Lower Arrow Lake, no. 24569, O.). The species has usually been mistaken for *S. longifolia* or *S. fluviatilis*, but apparently it forms with the southern var. *Bolanderiana* a well marked type in this section, and I am not yet sure to which other group of it *S. melanopsis* is most closely related. BALL (BOT. GAZ. **60**:51. 1915) speaks of a "*S. fluviatilis-melanopsis*" aggregation in contrast with the *S. sessilifolia* group, but I think *S. melanopsis* has very little to do with the true *S. fluviatilis*. The specimens from Umatilla County, Oregon, western slope of the Blue Mountains, in a swampy meadow at Ukiah, June 24, 1908, *W. Cusick* (nos. 3260, 3261, fr. juv.; N., St.), need further observation. The young fruits show a short style and are almost sessile. The main characters of *S. melanopsis* may be gathered from the key. The species is not even mentioned by ANDERSSON (1858, 1867, 1868), and its identity has first been revealed by ROWLEE (1900), who erroneously states that "it is particularly abundant along the Columbia River where NUTTALL saw it." I have not seen all the specimens cited by ROWLEE, but those of *Coville*, from Washington, Cowlitz County, north fork of Lewis River, July 16, 1898 (no. 719, fr.; W.), which are not mentioned in PIPER'S *Flora* and which have leaves that measure up to 9:2.2 cm., seem not to represent typical *S. melanopsis*, and I have not yet been able to identify them properly. In Herb. C. are similar specimens collected by *W. N. Suksdorf* in W. Klickitat County, "rocky bank of the Larm River," July 17, 1884. After all they may be taken for a form of *S. melanopsis* with very broad leaves. In California *S. melanopsis* is mostly represented by the following variety:

7b. S. MELANOPSIS var. **Bolanderiana,** nov. var.—*S. longifolia* Bebb in Watson, Bot. Calif. **2**:84. 1879, pro parte, non Muhl.; Jepson, Fl. Calif. **2**:340. 1909, pro parte; in Mem. Univ. Calif. **2**:178 (Silva Calif.). 1910, pro parte.—*S. Bolanderiana* Rowlee in Bull. Torr. Bot. Club **27**:257, *pl. 9, fig. 12*. 1900.—*S. exigua* var. *virens* Rowlee, l.c. 255, *pl. 9, fig. 11*.—*S. argophylla* Rowlee, l.c. 252, quoad specim. *Bolanderii* (non *Breweri!*) no. 5031.—*S. fluviatilis* Eastwood, Handb. Trees Calif. 37. 1905, pro parte, non Nutt.; Sudw.,

For. Trees Calif. Slope 222. *fig. 91.* 1908, pro parte.—Of this variety ROWLEE has given a very incomplete description, and in citing the specimens he says "*Bolander*, nos. 49, 58, 4958, 5031." There are no nos. 49 and 58 of *Bolander*, but only no. 4958, which has to be taken for the type. No. 5031 is also cited by ROWLEE under *S. exigua* var. *virens*, of which I previously have spoken, and again under *S. argophylla* as a number of *Brewer*, who, so far as I know, never collected a specimen bearing the same number at the same locality from which *Bolander's* plant came.

This variety differs from the type chiefly by the characters indicated in the key. ROWLEE's statement in his key that in *S. melanopsis* the leaves are "distinctly glaucous and prominently veiny beneath" while they are "not distinctly glaucous nor veiny beneath" in *S. Bolanderiana* is not correct. The leaves are sometimes rather greenish beneath in both forms. The typical form of var. *Bolanderiana* is somewhat pubescent, while most of the specimens before me belong to a glabrous form. There can also be observed a slight variation with partly hairy ovaries and fruits in the specimens of *J. Burtt Davy* (no. 5691, from Hoopa Valley, Humboldt County, California) and *S. Watson* (no. 1092, Truckee Valley, Washoe County, Nevada). Both need further observation, and may represent hybrids with *S. exigua*. This seems also the case with *A. A. Heller's* no. 6953 (along Coldstream, 3 miles above Truckee, July 17, 1908). On the other hand, specimens collected at Sunol Valley, Alameda County, June 29, 1916, by *L. R. Abrams* (no. 5692, no. 5693, f.; St.), of which the male plant cannot be distinguished from typical var. *Bolanderiana*, possess ovaries and fruits which are hairy throughout or become glabrous only to a slight degree. They do not look like hybrids, and seem to represent a distinct form with pubescent ovaries and rather silky tomentose young leaves.

The typical *S. Bolanderiana* has rather broad leaves, but there are before me many very narrow leaved specimens, and further observation in the field must show whether the forms with linear-lanceolate leaves can be separated from the typical form. I do not wish to propose too many new varieties and forms which are only known to me from herbarium specimens, but I believe that a

closer study of many difficult forms which I can only briefly mention will lead to a different conception of them.

I have seen specimens of var. *Bolanderiana* from the following counties in California (north to south): Humboldt, Siskiyou (*A. A. Heller*, no. 8058, female part not quite typical), Shasta, Lassen, Plumas, Butte, Nevada, ?Mendocino (*A. Kellogg* and *W. G. W. Hartford*, no. 922, ?Ukiah), Lake, Solano, Alameda (Sunol), Amador, Tuolumne, Mariposa (*Bolander*, no. 4958, type!, Yosemite Park, Slough's Valley), Fresno, Monterey, Tulare, and Kern. It may even occur farther south.

There is a specimen from San Bernardino County, near head of San Antonio Canyon, in a narrow rocky canyon, alt. 2250 m., July 5, 1918, *I. M. Johnston* (no. 2087, flor. abnorm. m. et f. mixtis; A.; "shrub, low, under 1 m."). The leaves are almost wholly glabrous when maturing, at least on the lower surface, which is more or less distinctly glaucescent. The flowers, however, are abnormal, the female ones hard to distinguish from those of *S. exigua*, but glabrous, or almost so. The form may belong to *S. exigua virens*, if there is really such a variety, or it may be related to var. *Bolanderiana*. The normal form is represented by *Johnston's* nos. 1401 and 1665, from the upper San Antonio Canyon. I am much obliged to Mr. JOHNSTON for the following information:

Numbers 1401, 1665, 2087 from near head of San Antonio Canyon. To me this is the most interesting plant I sent you. I have thoroughly explored the San Antonio Mountains, but I have only found the single colony from which all my specimens were obtained. It grows as a dense, low, compact shrub (hardly over a meter in height) on the rocky floor of a very deep gulch. A short distance away is found a large colony of *S. flavescens* and scattering shrubs of *S. Watsoni*. The nearest LONGIFOLIAE that I know of is 7 miles away and is the colony from which my 1685, which you doubtfully referred to *S. Parishiana*, was obtained. I have never yet seen in S. California a LONGIFOLIAE so high in the mountains and associating with such typically boreal species as this one does. You have probably noted that the aments contain both staminate and pistillate flowers, which may be due to its strange habitat. I noted that a large percentage of the aments were entirely sterile at the time I collected the specimens.

8. S. LONGIFOLIA Muhl. in Neue Schr. Ges. Natf. Fr. Berlin 4:238. *pl. 6. fig. 6.* 1803, non Lamarck;[11] in Ann. Bot. König

[11] According to the international rules, MUHLENBERG's name can stand because LAMARCK's (Fl. Fr. 2:232. 1778) is nothing but a synonym of *S. viminalis* L.; in following the Philadelphia Code the name *S. interior* Rowl. has to be used, and I would not keep MUHLENBERG's name if LAMARCK's were not an unconditional synonym, and could be applied to a form differing from typical *S. viminalis*.

2:66. *pl. 5. fig. 6.* 1806; Carey in Gray, Man. Bot. N.U.S. 429. 1848; Andersson in K. Sv. Vet.-Akad. Handl. **6**:54. *pl. 4. fig. 35.* 1867, pro parte et excl. var.; in DC., Prodr. **16**^{2}:214. 1868, pro parte et excl. var.; Bebb in Coult., Man. Bot. R. Mts. 335. 1885, pro parte; apud Watson and Coulter, Gray Man. ed. 6. 482. 1890; Robinson and Fernald, Gray's New Man. 323. *fig. 649.* 1908.—*S. fluviatilis* Sargent in Gard. and For. **8**:463. 1895, pro parte, non Nutt.; Silva N. Am. **9**:123. *pl. 474.* 1896, pro parte et excl. var.; Man. Trees N. Am. 175. 1905, pro parte; Schneider, Ill. Handb. Laubh. I. 32, *figs. 11 h–l, 12 m–m*^{1}. 1904; Ball in Proc. Iowa Ac. Sci. **7**:145. 1900; in Coult. and Nels., N. Man. R. Mts. Bot. 131. 1909, pro parte; in Bot. Gaz. **60**:397. 1915; Britt. and Brown, Ill. Fl. **1**:497. *fig. 1181.* 1896; Sudworth, Nomencl. Arb. Fl. U.S. 122. 1897, pro parte; Rydberg in Britt., Man. Fl. N. St. Can. 316. 1901; Hough, Handb. Trees N. St. Can. 84. *figs. 97, 98.* 1907, pro parte maxima.—*S. interior* Rowlee in Bull. Torr. Bot. Club **27**:253. *pl. 9, figs. 12, 13.* 1900; Small, Fl. S.E.U.S. 342. 1903, pro parte; Britt. and Shafer, N. Am. Trees 193. *fig. 154.* 1908; Britt. and Brown, Ill. Fl. ed. 2. **1**:595. *fig. 1458.* 1913; Rydberg, Fl. R. Mts. 192. 1917.—This is the type species of the section and the only one known from the central and northeastern states and eastern Canada. The type came from Lancaster, Pennsylvania. It has its headquarters in the regions of the Mississippi, Arkansas, and Missouri, while toward the east the Ohio seems to form the southern border line of its range up to Pennsylvania. The mouth of the Mississippi in Louisiana is the southernmost point of the range of *S. longifolia;* its western boundary runs apparently just south of the Red River in Louisiana and Texas, thence through western Kansas, the northeastern corner of Colorado, touching Wyoming in its northeastern part, from whence it runs through western Dakota to Manitoba. In Texas, southern New Mexico, and northwestern Mexico it is represented by var. *angustissima* (see later), while in the northwest from western Dakota and northeastern Wyoming through eastern Montana, Saskatchewan, and eastern Alberta the var. *pedicellata* seems to be the prevailing form, reaching its northwestern limit in the Yukon Valley (vicinity of Dawson and the adjacent parts of eastern Alaska, Fairbanks) and the upper Mackenzie region in the

Northwest Territories. The northern border line of the range of *S. longifolia* and var. *pedicellata* is not yet exactly known. Approximately it seems to run in the west from Fairbanks in Alaska to Fort Simpson in the Northwest Territories and through the Athabasca Plains and central (or southern?) Manitoba and southern Ontario to the south of James Bay and to about Lake St. Johns in Quebec, from where the eastern line turns southeast to western New Brunswick (Woodstock, Pokiok) and then southward to New Hampshire along the Connecticut River to Delaware and the District of Columbia.

The species apparently reaches its best development in the rich river bottoms from Louisiana to Indiana, while in Oklahoma, Kansas, Nebraska, and Iowa the form of the sand bars seems to prevail, which has narrower, smaller leaves. In the region of the Great Lakes and in the northeast, but also in other portions of the range under similar ecological conditions, the following variety seems to occur frequently:

S. LONGIFOLIA var. **Wheeleri,** nov. comb.—*S. interior* var. *Wheeleri* Rowlee in Bull. Torr. Bot. Club **27**:253, *pl. 9, fig. 14.* 1900. —*S. Wheeleri* Rydberg in Britt., Man. ed. 2. 1061. 1905; Britt. and Br., Ill. Fl. ed. 2. 1:595. 1913.—*S. longifolia* (vel *S. fluviatilis*) var. *argyrophylla* Auct. div. pro parte, non And.—I agree to a certain extent with SCHAFFNER (in Ohio Nat. **14**:255. 1914), who regards this variety as an ecological form, and I have already pointed out that similar forms seem to occur in *S. exigua* (see var. *luteo-sericea*), *S. melanopsis* var. *Bolanderiana*, etc. Those forms very often look quite distinct, especially in the herbarium. The broad leaved forms of var. *Wheeleri* can easily be taken for a well marked species if one does not have a very rich set of specimens showing all the intermediates between such forms as we know from Maine (Caribou) and New Brunswick and the narrow leaved forms from Lake Champlain, Lake Superior, etc. It may be that the easternmost forms are not quite identical with the typical var. *Wheeleri* from the region of the Great Lakes, but to decide this question we need a careful study of this form as it is observed in New Brunswick, Maine, Connecticut, western Quebec, and eastern Ontario. There is a male plant in cultivation in the Arnold

Arboretum which was brought by Professor *J. G. Jack* probably from the St. Lawrence region in Ontario. It is extremely like the female specimen of *Bissell* from Glastonbury, Connecticut, and both agree well with the specimens cited from Maine and New Brunswick. In *Bissell's* plant the stigmas are rather long and narrow, resembling somewhat those of the western *S. fluviatilis* but without a trace of a style. The leaves too of both plants are not very different in their shape, but var. *Wheeleri* has a coarser silky pubescence of longer hairs. ROWLEE stated that "the silvery vesture of this shrub is much like that of *S. argophylla* of the Pacific Coast." As I have explained under this species, ROWLEE did not interpret it correctly.

At present I refer to var. *Wheeleri* the following specimens, and I hope collectors will pay attention to this plant at the localities given.

Eastern North Dakota: Benson County, Pleasant Lake, 99 mer., everywhere along watercourses, July 2, 1911, *J. Lunell* (m., flor. satis abnorm.; A.).—Iowa: Story County, Ames, 1888, *A. S. Hitchcock* (m.; M.); Fremont County, Hamburg, July 4, 1914, *L. H. Pammel* and *H. B. Clarke* (no. 44, m.; A.; a hairy sand-bar form).—Illinois: St. Clair County, Cahokia, July 23, 1895, *N. M. Glatfelder* (m.; M.); Winnebago County, Fountaindale, 1877, *M. S. Bebb*, (fr.; M.; narrow leaved form, probably cultivated); Cook County, Dunning, fields, May 16, 1916, *F. C. Gates* (no. 1428, m.; C.).—Indiana: Noble County, near Rome City, June 11, 1916, *Deam* (no. 20118 A, ex parte, f., fr.; A.); Union County, Liberty, July 1886, *J. N. Rose* (st.; C).—Michigan: Wayne County, Belle Isle, July 8, 1903, *D. A. Farwell* (f.; A.; according to a letter of FARWELL this form was named by ROWLEE himself var. *Wheeleri*, but it represents a very glabrescent form difficult to separate from typical *longifolia*).—Wisconsin: Brown County, Green Bay, south shore, June 1878, *J. H. Schuette* (m., st.; C.).—Minnesota: Buffalo Lake, June 1891, *B. C. Taylor*, (m.; C.).—Ohio: Erie County, Cedar Point, August 2, 1895, *E. L. Moseley* (st.; G.); September 4, 1898, *Moseley* (st.; W.; folia ad 8:2 m. magna, elliptico-oblonga); July 3, 1908, *R. F. Griggs* (no. 2, m.; N.; folia ad 8:1.5 cm. magna, distanter ciliato-serrata); without exact locality and date, *W. S. Sullivant* (no. 49, st.; N.); Lake County, near Painesville, May 19, 1892, *O. Hacker* (no. 431, m.; C.); Franklin County, Columbus, 1840, *W. S. S.* (st.; G.); Ottawa County, Bay Point, sandy shore, August 20, 1914, *L. H. MacDaniels* and *A. J. Eames* (fr.); Ross County, Chillicothe, June 16, 1899, *A. D. Selby* (no. 120, st.; C.).—Pennsylvania: Erie County, Presque Isle, Lake Erie, July 23, 1868, *T. C. Porter* (st.; N., C.); York County, shores of the Susquehanna near

McCall's Ferry, September 13, 1864, *T. C. Porter* (m.; C.; "shrub 5–6 ft. high"; forma peculiaris foliis late oblongo-ellipticis ad 9:2.2 cm. magnis).—New York: Erie County, shores of Lake Erie near Buffalo, June 30, 1899, *J. F. Cowell* (st.; N.); Clinton County, shore of Lake Champlain, near Plattsburg, August 8, 1902, *A. Rehder* (st.; A.); Tompkins County, Fall Creek ravine, on rocks, May 29, June 6, 1885, *W. R. Dudley* (m., st.; C.; folia pl.m. oblanceolata).—Vermont: wet shore of Lake Champlain, July 8, 1914, *Ch. H. Knowlton* (m.; NE.); June 15, 1896, *A. J. Grout* (f.; NE.; stigmata satis elongata).—Connecticut: Hartford County, Glastonbury, banks of Connecticut River, May 18, 1902, *C. H. Bissell* (f.; G.; "small shrub"; forma distincta porro observanda); New London County, Lyme, near Selden's Cove, July 29, 1902, *C. B. Graves* (st.; G.; "2 ft. high"; ut praecedens).—Maine: Aroostook County, Caribou, gravelly river beach, July 18, 1902, *E. F. Williams, J. F. Collins,* and *M. L. Fernald* (st.; G.; forma satis distincta porro observanda); same locality and date, *E. F. Williams* (st.; A., G.).—New Brunswick: Woodstock, on the bars in the St. John River, August 30, 1899, *Macoun* (no. 22609, O.; st.; very much like the Connecticut forms); near Pokiok, July 8, 1889, *Brittain* (no. 24577, O.; st.; ut praecedens); above Fredericton, on island, August 23, 1890, *J. Brittain* (no. 6, fr.; C.; ut praecedens); Keswick, June 6, 1891, *J. Brittain* (no. 4, f.; C.).—Ontario: Lambton County, Fort Frank, 35 miles from Port Huron, Michigan, July 21, 1905, *C. K. Dodge* (st.; A.; forma densissime sericea); Welland County, Point Albino, August 28, 1896, *C. L. Pollard* (st.; W.); James Bay, Moose Factory, July 15, 1904, *W. Spreadborough* (no. 6262e, O.; st.; forma porro observanda paullo sericea).

Every species inhabiting such a wide area as *S. longifolia* and growing under so many different ecological conditions will naturally show a great degree of variability. Besides this there are quasi intermediate forms with *S. exigua* in all the regions where both species meet, and it is difficult to decide whether the northwestern forms of what I call var. *pedicellata* really belong to *S. longifolia* or to *S. exigua*, as BALL seems to believe according to his determinations in different herbaria. The synonymy of var. *pedicellata* may be given as follows:

8b. S. LONGIFOLIA var. PEDICELLATA Andersson in K. Sv. Vet.-Akad. Handl. **6**:55. 1867; in DC., Prodr. **16**^{2}:214. 1868.—*S. rubra* Richardson in Franklin, Narr. Jour. Polar Sea App. 752. 1823, nom. nud., non Hudson.—*S. longifolia* (?) Torrey in Ann. Lyc. Nat. Hist. N.Y. **2**:248 (Coll. Pl. R. Mts. James).[12] 1828; Andersson in

[12] The specimen (preserved in N.) has been collected by *James* either in eastern Wyoming or eastern Colorado, and seems to belong to this variety.

Öfv. K. Vet.-Akad. Förh. **15**:116. 1858, ex parte; Macoun, Cat. Canad. Pl. 450. 1883, ex parte; Sargent, Rep. For. Trees N. Am. 10th Census U.S. **9**:168. 1884, ex parte.—*S. fluviatilis* Sargent in Gard. and For. **8**:463. 1895, ex parte, non Nutt.; Rowlee in Bull. Torr. Bot. Club **27**:254. 1900, ex parte; Henry, Fl. S. Br. Col. 97. 1915.—*S. interior* Rowlee, l.c., 253, ex parte; Britt. and Br., Ill. Fl. **1**:595. 1913, ex parte.—*S. linearifolia* Rydbg. in Britton, Man. 316. 1901; Fl. Color. 94. 1906, ex parte; Fl. R. Mts. 192. 1917, ex parte; Small, Fl. S.E.U.S. 342. 1903, ex parte.—*S. longifolia* var. *interior* Jones, Willow Fam. 25. 1908, ex parte.—I have seen a photograph and fragments of the type of var. *pedicellata*, collected by *E. Bourgeau*, "Saskatchewan bords des Lacs, abondant, 21 Juin 1858" and preserved in Herb. K., and also of the type of *S. rubra* Rich. from the "Mackenzie River." This specimen of *Richardson's* represents the same form as the material from "Cumberland House" in Saskatchewan, which is a syntype of *S. linearifolia* Rydbg. in Herb. N. This variety differs from typical *S. longifolia* chiefly in its narrower, linear leaves, and its glabrous ovaries, which are more or less sessile when young but usually distinctly pediceled when in fruit, the pedicels often being twice as long as the ventral gland. As previously stated, var. *pedicellata* is the prevailing form in the northwestern part of the range of *S. longifolia*, but there are also forms near the southern limit of its habitat which can hardly be distinguished from var. *pedicellata* (for instance *Munson's* specimens from the Red River near Colbert's Ferry, north of Denison, Texas, April 19, 1911, f., fr.; A.).

As previously stated, the most southern form of *S. longifolia* is represented by var. *angustissima* And. (1858[13]) with which I have dealt in Bot. Gaz. **65**:26. 1918. Besides the Mexican specimens here cited, I refer the following to this variety, which seems too closely connected with the typical *S. longifolia* to be kept as a distinct species.

Specimens examined.—Texas: without exact locality and date, *Berlandier* (nos. 911, 2341, 2368, 3019, cotypes; G., M.; 1938, f.; M.; nos. 2341 and

[13] Later, in Monogr. 1867 and in Prodr. 1868, Andersson used this name for different forms, partly belonging to *S. sessilifolia* var. *Hindsiana*, partly to *S. exigua* (probably var. *stenophylla*).

2368, of which the last has to be taken as the type of *S. Thurberi*, have been erroneously attributed by ROWLEE to *G. Thurber*, to whom only the following specimen belongs); Horse Head Cruping (?) River, October 1850, *G. Thurber* (no. 95; G.; "10–12 ft."); ?Pecos County, banks of the Pecos, 1889, *Nealhy* (no. 33, m.; W.); September 1881, *V. Havard* (m., f.; W.; ad var. *typicam* accedens); Brewster County, Rio Grande, south of Chisos Mountains, August 1883, *V. Havard* (m., f.; W.); Val Verde County, Del Rio, along streams, October 18, 1916, *E. J. Palmer* (no. 11069, f.; A.); Potter County, Amarillo, creek banks, July 13, 1917, *E. J. Palmer* (no. 12539, f., fr.; A.; ad var. typicam accedens); along Rio Grande, near San Vincente, August 26, 1915, *M. S. Young* (m., f.; M.); Guadalupe County, in the dry bed of the Cibolo 12 miles east of New Braunfels, August 1851, *F. Lindheimer* (no. 615 [=1191], f.; G., M.); Comanche County, Comanche Spring, *Lindheimer* (no. 1190, f.; M.); Matagorda County, banks of Peyton Creek near Bay City, May 6, 1916, *E. J. Palmer* (no. 9689, m.; A.); Cameron County, near Brownsville, November 1888, *Nealhy* (no. 30, f., fr.; W.); (New Mexico?), Rio Grande, July 1848, *C. Wright* (m.; G.; "small tree"); without locality, 1849, *C. Wright* (no. 668, m.; G., W.).

There have also been described the following forms which I have not yet been able to elucidate: *S. longifolia* var. *sericans* Nees v. Esenbeck in Wied-Neuwied, Reise In. N. Am. **2**:448. 1841; Engl. ed. by Lloyd, Trav. Int. N.A. 518. 1843, collected on the Missouri, probably in eastern Montana about July 8 (see *l.c.* **1**:472 [Engl. ed. p. 211]). I would refer it to *S. exigua*, but the lower flowers of the male aments are described as "triandri"; otherwise the description agrees with *S. exigua*.—*S. longifolia* f. *integerrima* Kuntze, Rev. Gen. Pl. **2**:643. 1891, and f. *paucidenticulata* Kuntze, l.c. The first is characterized by the phrase "folia denticulata" and as type is given "U. St., Madisonthal"; while the second has "folia paucidenticulata" and came from "Cheyenne, Nebr." The author adds "Ausserdem kann man eine f. *multidenticulata* unterscheiden." I suppose those forms are simply typical *S. longifolia*.

With the hybrids which doubtless occur only too frequently where different species grow together it is impossible to deal, as long as it has not yet been possible to limit the species in a more satisfactory manner. The main purpose of this paper is to point out the correct application of certain names, and to direct attention to such forms as need a close study in the field.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

RESPIRATION AFTER DEATH[1]

A. R. C. HAAS

(WITH THREE FIGURES)

It is commonly stated that when respiration ceases the protoplasm is no longer alive, but it is uncertain in most cases whether respiration ends as soon as death occurs or whether it continues for some time afterward.

It was stated by JOHANNSEN (8), by DETMER (6), and by PFEFFER (17) that in general there is no production of CO_2 after death, although REINKE (18) and BRENSTEIN (3) held the opposite view. BUCHNER (4) showed that yeast which had been treated with acetone and ether and which was incapable of cell division, and in all probability dead, could produce CO_2 by fermentation. KOSTYTSCHEFF (10) found that an aërobic plant, *Aspergillus niger*, treated in this manner was still capable of respiration. Since some of the cells appeared to be alive after the treatment, he used heat to kill them. After this the oxidation was extremely small. This is to be expected as the oxidases are, for the most part, injured or destroyed by heat. Similar experiments have been made on bacteria. WARBURG (21) obtained a completely sterile acetone preparation of *Staphylococcus* which respired about one thirty-sixth as much as the living material. WARBURG and MEYERHOF (21) found that treatment with acetone and ether had little effect on the consumption of oxygen by unfertilized sea urchin eggs (although they were completely killed), but the same treatment diminished the consumption of oxygen by fertilized eggs by 90 per cent.

Numerous experiments have been made with cells killed by mechanical means (finely ground) or by freezing and thawing. PALLADIN (16) found that finely ground wheat produced less CO_2 than the normal amount, while various plants exposed to $-20°$ C. for some time and then thawed out showed a loss of power to absorb oxygen, but continued to produce CO_2.

[1] A preliminary communication appeared in Proc. Nat. Acad. Sci.

BATELLI and STERN (2) have found oxidation in finely ground tissue and watery extracts. Their results have been criticized by WARBURG.

WARBURG (21) found that the finely ground red blood corpuscles of birds consumed less oxygen than intact cells. Unfertilized sea urchin eggs, cytolyzed in distilled water, consumed as much oxygen as the intact eggs but produced no CO_2. In fertilized eggs cytolysis reduced the oxygen consumption by 90 per cent or more. A fuller account of the literature seems unnecessary, as it has been summarized by WARBURG.

It will be noticed that in the cases previously reported respiration after death is greatly reduced or entirely lacking. The only instance in which post mortem respiration is greater than in normal tissue is that reported by LOEB and WASTENEYS (11), in which unfertilized sea urchin eggs, cytolyzed by saponin, showed from 3 to 7 times the normal rate of respiration. It is of considerable interest therefore to find that the respiration of *Laminaria* after death may be much greater than when in its normal condition.

The determination of the output of CO_2 was made in the following manner. The increase in the hydrogen ion concentration of sea water containing pieces of *Laminaria* (in the dark) served as a measure of the respiration of the tissue. The decrease in PH value was determined by the addition of a suitable indicator (phenolsulphone phthalein) by comparing the colors with those of a series of buffer mixtures containing an equal amount of the same indicator.

Each piece of *Laminaria* was kept for about half an hour in sea water before beginning the experiment. This treatment tended to obviate any effects of the slight wounding (19, 20). The material was then rolled into a scroll and inserted into a Pyrex glass tube (7) fused shut at one end and attached to a paraffined rubber tube at the open end. Sea water, of the temperature of the bath (16 ± 1°C.), was placed in the tube and the latter inserted in a black enameled collapsible tin tube in the bath. The sea water surrounding the tissue (in the tube) was renewed several times before beginning the experiment. A definite amount of sea water

(6 cc.) was placed in each of the tubes. The tubes were clamped shut in such a way as to include a very small air bubble (always of the same size) which served as a stirrer. This was sufficiently accurate and was more convenient than paraffined glass beads. After a tube had been in the dark at 16± 1°C. for a definite period, it was removed from the bath, and the contents shaken by inverting the tube several times. The sea water was then poured rapidly into an empty tube of equal diameter, to which the same quantity (3 drops of 0.01 per cent aqueous phenolsulphone phthalein to 6 cc. of solution) of indicator was added as had been added to the buffer mixtures. The color was then compared with the colors of a series of buffer mixtures by the use of a constant source of light (the "Daylight" lamp) and the PH value determined. The same amount of sea water was again added to the tissue in the tube and the tube exposed (at 16± 1°C. in the dark) for the same length of time as before, after which it was removed from the bath and the PH value again determined. This was repeated until the respiration in sea water was approximately constant. Then sea water containing the killing agent was substituted for the sea water, and the PH values determined as before after a series of successive periods (each of the same length as the original).

In some cases (acetone 17.4 and alcohol 24.2 per cent) the killing agent extracted from the plant a small amount of pigment which interfered with the color of the indicator.[2] This difficulty disappeared after the first two periods, however, as was shown by running pure hydrogen through the solution, after which it returned to the color found in normal sea water containing indicator. This method also showed conclusively that the only acid excreted by the plant was carbonic acid.

The methods of killing the tissue were various. Sea water containing anesthetics (made up to the conductivity of sea water by the addition of concentrated sea water) was employed in many of the experiments. In this case the respiration was determined for several periods of equal length in sea water (the solution being renewed after each period). The sea water was then replaced by

[2] This did not occur with low concentrations of these substances.

sea water containing anesthetic and the respiration determined after successive equal periods until death ensued, and for some time thereafter.

TABLE I

CONTROL FOR I A: 7 PERIODS (27.5 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.1−7.6 =0.5	0.5	0.5	1.00	27.5	0.5 ÷0.5=1.00
2.....	8.1−7.6 =0.5	1.0	1.0	1.00	55.0	0.5 ÷0.5=1.00
3.....	8.1−7.6 =0.5	1.5	1.5	1.00	82.5	0.5 ÷0.5=1.00
4.....	8.1−7.6 =0.5	2.0	2.0	1.00	110.0	0.5 ÷0.5=1.00
5.....	8.1−7.65=0.45	2.45	2.5	0.98	137.5	0.45÷0.5=0.90
6.....	8.1−7.65=0.45	2.90	3.0	0.96	165.0	0.45÷0.5=0.90
7.....	8.1−7.65=0.45	3.35	3.5	0.96	192.5	0.45÷0.5=0.90

TABLE I A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (22 MIN. EACH) IN SEA WATER AND DURING 7 SUBSEQUENT PERIODS IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Period	Change in PH	Change in PH during saturation with ethyl bromide = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time during saturation with ethyl bromide in min.	Relative rate of respiration
Sea water.....	1	8.1 −7.8 =0.30	.....	.....	.....	22	0	.....
" "	2	8.1 −7.8 =0.30	.....	.....	.....	44	0	.....
Sea water containing ethyl bromide.....	3	7.32−6.16*=1.16+	1.16	0.30	3.8	66	22	1.16÷0.30=3.8
Sea water containing ethyl bromide.....	4	7.32−6.16*=1.16+	2.32	0.60	3.8	88	44	1.16÷0.30=3.8
Sea water containing ethyl bromide.....	5	7.32−6.69 =0.63	2.95	0.90	3.2	110	66	0.63÷0.30=2.1
Sea water containing ethyl bromide.....	6	7.32−7.07 =0.25	3.20	1.20	2.6	132	88	0.25÷0.30=0.8
Sea water containing ethyl bromide.....	7	7.32−7.21 =0.11	3.31	1.50	2.2	154	110	0.11÷0.30=0.4
Sea water containing ethyl bromide.....	8	7.32−7.32 =0.0	3.31	1.80	1.8	176	132	0.0 ÷0.30=0.0

* Approximate (at this point indicator is not very sensitive to slight changes in acidity).

As it was important to know the time of death as accurately as possible, determinations of the electrical conductivity of the tissue were made by the method of OSTERHOUT (12, 13). If the

electrical resistance of the normal tissue be called 100 per cent, it is found that after killing the resistance falls to about 10 per cent. When the resistance has fallen to 15 per cent the tissue is for all practical purposes dead, as there is no recovery when it is returned to normal conditions.[3]

The results of the experiments showing the relative amount and relative rate of respiration of tissue of *Laminaria* when subjected to various treatments are presented in tabular form. In every case 6–12 or more closely agreeing results were obtained and the data

TABLE I B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 20° C.

SEA WATER		SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE	
Time in min.	Percentage net resistance	Time in min.	Percentage net resistance
0.....	100	0.....	100
100.....	90.9	1.....	105
200.....	87.8	5.....	79
		10.....	56
		15.....	39
		20.....	30
		35.....	16
		60.....	11
		80.....	10
		1000.....	5

of a typical case presented. Table I A shows the relative amount and relative rate of respiration as influenced by sea water approximately saturated with ethyl bromide. This is the only experiment in which the PH value of the control differed from the PH value of the sea water containing anesthetic (at the start of the experiment). It will be noted that after about 130 minutes no respiration was detected. An examination of table I B, which gives the net electrical resistance of *Laminaria*, shows that the material can be considered dead before the end of 60 minutes. From table I A it is seen that at the end of 60 minutes the relative rate and relative

[3] The determinations were made in part by Professor OSTERHOUT and in part by me.

amount of respiration are approximately double that of the normal, although the tissue is shown by the method of electrical resistance to be dead. This is brought out very strikingly in fig. 1 where we plot as ordinates the relative amount of respiration (curve C, table I A), relative rate of respiration (curve B, table I A), net resistance as percentage of that at the start (curve A, table I B) respectively (unbroken lines). When the relative rate of respiration has practically reached zero (curve B) the relative amount of

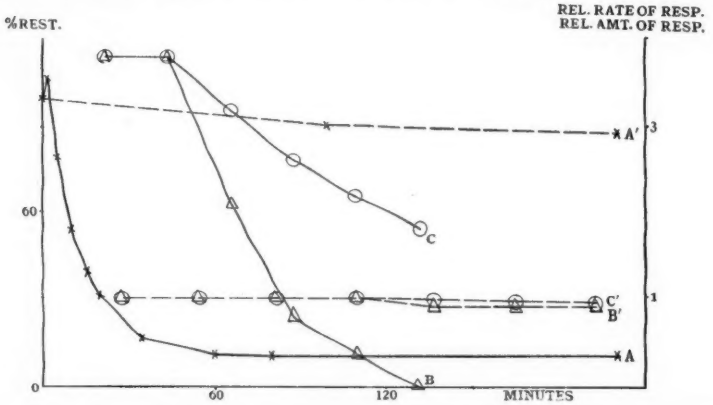

FIG. 1.—Curves showing effect produced by sea water, approximately saturated with ethyl bromide, upon relative amount and relative rate of respiration, and upon net electrical resistance of *Laminaria:* curve A, ordinates represent net resistance as percentage of that at start; curve B, ordinates represent relative rate of respiration; curve C, ordinates represent relative amount of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

respiration is above unity. At the end of 60 minutes, when the tissue can be considered dead, the relative rate is seen to be about double that of the normal rate.

Table II A shows the effect produced by sea water containing 17.4 per cent (by volume) acetone, made up to the electrical conductivity of sea water by the addition of concentrated sea water, upon the relative amount and relative rate of respiration of *Laminaria.* At the end of 2.5 hours the rate of respiration is still above the normal rate, while the relative amount of respira-

tion is nearly 2. Table II B shows the net electrical resistance. It is seen that the material is dead before the end of 100 minutes.

TABLE II

CONTROL FOR II A: 9 PERIODS (21 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.37−7.67=0.70	0.70	0.70	1.0	21	0.70÷0.70=1.0
2.....	8.37−7.67=0.70	1.40	1.40	1.0	42	0.70÷0.70=1.0
3.....	8.37−7.70=0.67	2.07	2.10	0.99	63	0.67÷0.70=0.95
4.....	8.37−7.73=0.64	2.71	2.80	0.97	84	0.64÷0.70=0.91
5.....	8.37−7.73=0.64	3.35	3.50	0.96	105	0.64÷0.70=0.91
6.....	8.37−7.73=0.64	3.99	4.20	0.95	126	0.64÷0.70=0.91
7.....	8.37−7.72=0.65	4.64	4.90	0.95	147	0.65÷0.70=0.93
8.....	8.37−7.73=0.64	5.28	5.60	0.94	168	0.64÷0.70=0.91
9.....	8.37−7.72=0.65	5.93	6.30	0.94	189	0.65÷0.70=0.93

TABLE II A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (24 MIN. EACH) IN SEA WATER, AND DURING 7 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 17.4 PER CENT (BY VOLUME) OF ACETONE

Solution	Period	Change in PH	Change in PH during exposure to acetone = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time of exposure to acetone in min.	Relative rate of respiration
Sea water.....	1	8.37−7.76=0.61	.....	.....	.....	24	0	.....
" "	2	8.37−7.78=0.59	.....	.....	.....	48	0	.....
Sea water containing 17.4 per cent acetone	3	8.37−6.80=1.57	1.57	0.60	2.61	72	24	1.57÷0.60=2.6
Sea water containing 17.4 per cent acetone	4	8.37−6.75=1.62	3.19	1.20	2.66	96	48	1.62÷0.60=2.7
Sea water containing 17.4 per cent acetone	5	8.37−7.06=1.31	4.50	1.80	2.50	120	72	1.31÷0.60=2.2
Sea water containing 17.4 per cent acetone	6	8.37−7.50=0.87	5.37	2.40	2.24	144	96	0.87÷0.60=1.5
Sea water containing 17.4 per cent acetone	7	8.37−7.54=0.83	6.20	3.00	2.07	168	120	0.83÷0.60=1.4
Sea water containing 17.4 per cent acetone	8	8.37−7.62=0.75	6.95	3.60	1.93	192	144	0.75÷0.60=1.3
Sea water containing 17.4 per cent acetone	9	8.37−7.74=0.63	7.58	4.20	1.80	216	168	0.63÷0.60=1.1

The fact that respiration proceeds here at a rate much above the normal (although death has taken place) is very clearly brought

out by comparing the curves for table II A and II B as given in fig. 2. The ordinates represent relative amount of respiration (curve A, table II A), relative rate of respiration (curve C, table II A), net resistance as percentage of that at the start (curve B, table II B) respectively (unbroken lines). The relative rate and relative amount of respiration at the end of over 2.5 hours are still much above the normal even though the measurements of electrical resistance have shown the tissue to be dead before 100 minutes.

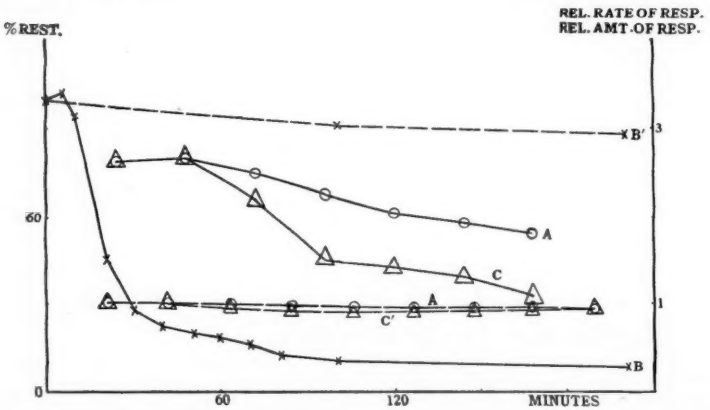

FIG. 2.—Curves showing effect produced by sea water containing 17.4 per cent (by volume) of acetone upon relative amount and relative rate of respiration, and effect produced by sea water containing 16.2 per cent of acetone upon net electrical resistance of *Laminaria:* curve A, ordinates represent relative amount of respiration; curve B, ordinates represent net resistance as percentage of that at start; curve C, ordinates represent relative rate of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

Table III A shows the effect produced by sea water containing 24.2 per cent (by volume) of ethyl alcohol (made up to conductivity of sea water by the addition of concentrated sea water). In fig. 3 the ordinates represent: relative amount of respiration (curve A, table III A), relative rate of respiration (curve C, table III A), net resistance as percentage of that at the start (curve B, table III B) respectively (unbroken lines). If we consider the material dead at the end of 90 minutes, we find that the

rate of respiration is much above the normal rate, while the relative amount of respiration is above 2.

TABLE II B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER CONTAINING 16.2 PER CENT (BY VOLUME) OF ACETONE, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 15.4° C.

SEA WATER		SEA WATER CONTAINING 16.2 PER CENT ACETONE	
Time in min.	Percentage net resistance	Time in min.	Percentage net resistance
0.....	100	0.....	100
100.....	90.9	5.....	105.5
200.....	87.8	10.....	94.3
		20.....	46.6
		30.....	28.4
		40.....	22.7
		50.....	20.2
		60.....	18.2
		70.....	16.2
		80.....	13.2
		100.....	11.1
		200.....	8.7
		300.....	6.7

TABLE III

CONTROL FOR III A: 8 PERIODS (30.25 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	7.90−7.53=0.37	0.37	0.37	1.0	30.25	0.37÷0.37=1.0
2.....	7.90−7.53=0.37	0.74	0.74	1.0	60.50	0.37÷0.37=1.0
3.....	7.90−7.53=0.37	1.11	1.11	1.0	90.75	0.37÷0.37=1.0
4.....	7.90−7.54=0.36	1.47	1.48	0.99	121.00	0.36÷0.37=0.97
5.....	7.90−7.54=0.36	1.83	1.85	0.99	151.25	0.36÷0.37=0.97
6.....	7.90−7.55=0.35	2.18	2.22	0.98	181.50	0.35÷0.37=0.95
7.....	7.90−7.55=0.35	2.53	2.59	0.97	211.75	0.35÷0.37=0.95
8.....	7.90−7.58=0.32	2.85	2.96	0.96	242.00	0.32÷0.37=0.87

Table IV shows the effect produced upon the relative amount and relative rate of respiration of *Laminaria* by sea water containing 3.2 per cent (by volume) of formaldehyde. The solution was

made up to the conductivity of sea water by the addition of concentrated sea water. The free acid of the formaldehyde was first neutralized by the addition of a little sodium carbonate. This is allowable for the purposes of the present investigation, for its effect would be to make the amount of CO_2 produced appear somewhat less than was actually the case. At the end of 4 hours the relative rate of respiration was still above the normal, while the

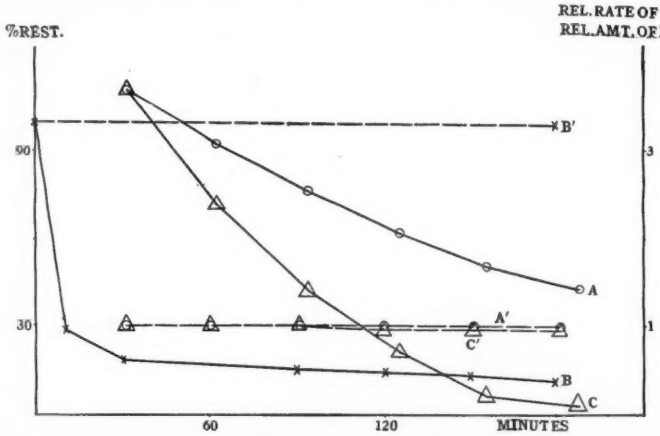

FIG. 3.—Curves showing effect produced by sea water containing 24.2 per cent (by volume) of ethyl alcohol upon relative amount and relative rate of respiration and upon net electrical resistance of *Laminaria:* curve A, ordinates represent relative amount of respiration; curve B, ordinates represent net resistance as percentage of that at start; curve C, ordinates represent relative rate of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

relative amount of respiration was much above the normal. At this concentration of formaldehyde *Laminaria* is practically dead in 180 minutes. In table IV, however, after 280 minutes the relative rate of respiration of *Laminaria* is still above normal, while at 180 minutes the relative rate is far above normal.

For purposes of comparison other methods of killing were tried. By making preliminary conductivity experiments with *Laminaria*, it was found that when it is dried upon cheesecloth in the sunlight in a current of dry air, we can consider the tissue practically dead

in 135 minutes. After such treatment the material becomes green. It was placed in sea water for 14 minutes; it lost its crispness and

TABLE III A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (31.25 MIN. EACH) IN SEA WATER AND DURING 6 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 24.2 PER CENT (BY VOLUME) OF ETHYL ALCOHOL

Solution	Period	Change in PH	Change in PH during the 6 later periods = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure during the 6 later periods	Relative rate of respiration
Sea water	1	7.90−7.43 =0.47	.....	.....	.....	31.25	0	.....
" "	2	7.90−7.43 =0.47	.....	.....	.....	62.50	0	.....
Sea water containing 24.2 per cent ethyl alcohol	3	7.90−6.16*=1.74	1.74	0.47	3.57	93.75	31.25	1.74÷0.47=3.7
Sea water containing 24.2 per cent ethyl alcohol	4	7.90−6.75 =1.15	2.89	0.94	3.07	125.00	62.50	1.15÷0.47=2.4
Sea water containing 24.2 per cent ethyl alcohol	5	7.90−7.25 =0.65	3.54	1.41	2.51	156.25	93.75	0.65÷0.47=1.4
Sea water containing 24.2 per cent ethyl alcohol	6	7.90−7.55 =0.35	3.89	1.88	2.07	187.50	125.00	0.35÷0.47=0.7
Sea water containing 24.2 per cent ethyl alcohol	7	7.90−7.80 =0.10	3.99	2.35	1.69	218.75	156.25	0.10÷0.47=0.2
Sea water containing 24.2 per cent ethyl alcohol	8	7.90−7.84 =0.06	4.05	2.82	1.43	250.00	187.50	0.06÷0.47=0.1

* Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE III B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER CONTAINING 24 PER CENT (BY VOLUME) OF ETHYL ALCOHOL, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 13.3° C.

SEA WATER		SEA WATER CONTAINING 24 PER CENT ETHYL ALCOHOL	
Time in min.	Percentage net resistance	Time in min.	Percentage net resistance
0.....	100	0.....	100
240.....	98	10.....	28.8
		30.....	18.6
		90.....	15.2
		120.....	14.9
		150.....	13.5
		180.....	11.8
		210.....	11.3

became flaccid. The relative amount and relative rate of respiration of pieces of *Laminaria* were determined before and after the

drying treatment (which killed the tissue). The results are given in table V A, the control data being given in table V. The relative amount of respiration after the treatment was very high and after 2 hours was still at 3. The relative rate of respiration after the treatment was very high at the start but gradually declined to normal in 2 hours.

TABLE IV

CONTROL FOR IV A: 15 PERIODS (21 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.37−7.80=0.57	0.57	0.52	1.1	21	0.57÷0.52=1.10
2.....	8.37−7.90=0.47	1.04	1.04	1.0	42	0.47÷0.52=0.90
3.....	8.37−7.90=0.47	1.51	1.56	0.96	63	0.47÷0.52=0.90
4.....	8.37−7.90=0.47	1.98	2.08	0.95	84	0.47÷0.52=0.90
5.....	8.37−7.92=0.45	2.43	2.60	0.93	105	0.45÷0.52=0.87
6.....	8.37−7.92=0.45	2.88	3.12	0.92	126	0.45÷0.52=0.87
7.....	8.37−7.92=0.45	3.33	3.64	0.92	147	0.45÷0.52=0.87
8.....	8.37−7.92=0.45	3.78	4.16	0.90	168	0.45÷0.52=0.87
9.....	8.37−7.90=0.47	4.25	4.68	0.90	189	0.47÷0.52=0.90
10.....	8.37−7.90=0.47	4.72	5.20	0.91	210	0.47÷0.52=0.90
11.....	8.27−7.90=0.47	5.19	5.72	0.91	231	0.47÷0.52=0.90
12.....	8.37−7.90=0.47	5.66	6.24	0.91	252	0.47÷0.52=0.90
13.....	8.37−7.90=0.47	6.13	6.76	0.91	273	0.47÷0.52=0.90
14.....	8.37−7.90=0.47	6.60	7.28	0.91	294	0.47÷0.52=0.90
15.....	8.37−7.90=0.47	7.07	7.80	0.91	315	0.47÷0.52=0.90

Another method used in killing the tissue was by placing it in running tap water. A preliminary determination of the electrical resistance showed that 22 hours were more than sufficient to kill the tissue. The experiment was begun at 11:18 A.M. and at 7:50 P.M. the tissue was still somewhat alive, but at 9:25 A.M. next day the tissue had in all probability been dead for some time. The respiration was then determined before and after exposure to running tap water for 19 hours. The results are given in table VI, the data for the control being given in table V A. In table VI it is obvious that no respiration of the tissue was observable after it had been in tap water for 19 hours. There is of course the possibility that the rise and decline of the respiration after death was so rapid as to escape observation if the tissue had been dead much before the end of 19 hours.

TABLE IV A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (23.5 MIN. EACH) IN SEA WATER AND DURING 13 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 3.2 PER CENT FORMALDEHYDE

Solution	Period	Change in PH	Change in PH during exposure to formaldehyde = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time of exposure to formaldehyde in min.	Relative rate of respiration
Sea water	1	8.37−7.82=0.55	.....	.....	.....	23.5	0	.....
" "	2	8.37−7.82=0.55	.....	.....	.....	47.0	0	.....
Sea water containing 3.2 per cent formaldehyde	3	8.37−6.90=1.47	1.47	0.55	2.67	70.5	23.5	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	4	8.37−6.90=1.47	2.94	1.10	2.67	94.0	47.0	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	5	8.37−7.05=1.32	4.26	1.65	2.58	117.5	70.5	1.32÷0.55=2.4
Sea water containing 3.2 per cent formaldehyde	6	8.37−7.15=1.22	5.48	2.20	2.49	141.0	94.0	1.22÷0.55=2.2
Sea water containing 3.2 per cent formaldehyde	7	8.37−7.33=1.04	6.52	2.75	2.37	164.5	117.5	1.04÷0.55=1.9
Sea water containing 3.2 per cent formaldehyde	8	8.37−7.50=0.87	7.39	3.30	2.24	188.0	141.0	0.87÷0.55=1.6
Sea water containing 3.2 per cent formaldehyde	9	8.37−7.55=0.82	8.21	3.85	2.13	211.5	164.5	0.82÷0.55=1.5
Sea water containing 3.2 per cent formaldehyde	10	8.37−7.58=0.79	9.00	4.40	2.04	235.0	188.0	0.79÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	11	8.37−7.60=0.77	9.77	4.95	1.97	258.5	211.5	0.77÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	12	8.37−7.65=0.72	10.49	5.50	1.90	282.0	235.0	0.72÷0.55=1.3
Sea water containing 3.2 per cent formaldehyde	13	8.37−7.70=0.67	11.16	6.05	1.84	305.5	258.5	0.67÷0.55=1.2
Sea water containing 3.2 per cent formaldehyde	14	8.37−7.80=0.57	11.73	6.60	1.77	329.0	282.0	0.57÷0.55=1.0
Sea water containing 3.2 per cent formaldehyde	15	8.37−7.85=0.52	12.25	7.15	1.71	352.5	305.5	0.52÷0.55=0.95

TABLE V

CONTROL FOR V A: 3 PERIODS (25.75 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS AN INTERVAL OF 19 HOURS DURING WHICH TISSUE WAS BATHED IN RUNNING SEA WATER

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.0−7.58=0.42	0.42	0.42	1.00	25.75	0.42÷0.42=1.00
2.....	8.0−7.58=0.42	0.84	0.84	1.00	51.50	0.42÷0.42=1.00
3.....	8.0−7.60=0.40	1.24	1.26	0.98	77.25	0.40÷0.42=0.95

By determining the electrical resistance it was found that *Laminaria* is killed by exposure to 35° C. for 70 minutes. The respiration before and after such exposure was then determined. During the treatment at 35° C. the material was removed from the tubes and placed in a large volume of the sea water kept at 35° C. The results are given in table VII A. After the exposure to 35° C., the relative amount and rate of respiration had fallen considerably below the normal. This might be expected on the ground that oxidizing enzymes are injured or destroyed by heat.

TABLE V A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 6 PERIODS (30.5 MIN. EACH) IN SEA WATER; AT END OF SECOND PERIOD MATERIAL DRIED IN CURRENT OF AIR IN SUN FOR 139 MINUTES; MATERIAL THEN PLACED IN SEA WATER AT 22° C. FOR 15 MINUTES BEFORE BEGINNING THIRD PERIOD.

Period	Change in PH	Change in PH after drying = A	Change in PH before drying, calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in. min.) of exposure after drying	Relative rate of respiration
1.....	8.15−8.0 =0.15	.....	.....	.....	30.5	0	.....
2.....	8.15−8.0 =0.15	.....	.....	.....	61.0	0	.....
3.....	8.15−7.20=0.95	0.95	0.15	6.3	91.5	30.5	0.95÷0.15=6.3
4.....	8.15−7.70=0.45	1.40	0.30	4.6	122.0	61.0	0.45÷0.15=3.0
5.....	8.15−7.85=0.30	1.70	0.45	3.8	152.5	91.5	0.30÷0.15=2.0
6.....	8.15−8.00=0.15	1.85	0.60	3.1	183.0	122.0	0.15÷0.15=1.0

It is well known that severe injury causes a considerable rise in the respiration, and it seemed desirable to make such experiments with *Laminaria*. After the normal respiration of a piece of tissue had been determined, the material was removed from the tube and finely macerated (by means of the jagged end of a tube of Pyrex glass) on a piece of tested filter paper. The minced *Laminaria* was put back into the tube and rinsed 6–10 times with sea water until none of the liberated pigment could be distinguished in the sea water. Fresh sea water was then added and the respiration determined. The results are given in table VIII, the control data being given in table V. In table VIII it will be observed that the relative amount and relative rate of respiration are both

TABLE VI A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 3 PERIODS (23 MIN. EACH) IN SEA WATER; AT END OF SECOND PERIOD MATERIAL PLACED IN RUNNING TAP WATER FOR 19 HOURS; MATERIAL THEN PLACED IN SEA WATER AT 22° C. FOR 34 MINUTES BEFORE BEGINNING THIRD PERIOD

Period	Change in PH	Change in PH after exposure to tap water = A	Change in PH before exposure to tap water, calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure after exposure to tap water	Relative rate of respiration
1.....	8.0−7.7=0.3	.....	.....	.....	23	0	.....
2.....	8.0−7.7=0.3	.....	.....	.....	46	0	.....
3.....	8.0−8.0=0.0	0	0.3	0	69	23	0.0÷0.3=0.0

TABLE VII

CONTROL FOR VII A: 4 PERIODS (25.5 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL KEPT IN SEA WATER AT 16° C. FOR 70 MINUTES

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.0−7.57=0.43	0.43	0.425	1.01	25.5	0.43÷0.425=1.01
2.....	8.0−7.58=0.42	0.85	0.85	1.00	51.0	0.42÷0.425=0.99
3.....	8.0−7.58=0.42	1.27	1.275	1.00	76.5	0.42÷0.425=0.99
4.....	8.0−7.58=0.42	1.69	1.70	0.99	102.0	0.42÷0.425=0.99

TABLE VII A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 4 PERIODS (28 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL PLACED IN LARGE VOLUME OF SEA WATER AND KEPT AT 35° C. FOR 70 MINUTES

Period	Change in PH	Change in PH after exposure following second period = A	Change in PH immediately after second period = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure during last two periods	Relative rate of respiration
1.....	8.0−7.25=0.75	.....	.....	.....	28	.....	.....
2.....	8.0−7.28=0.72	.....	.....	.....	56	.....	.....
3.....	8.0−7.80=0.20	0.20	0.735	0.27	84	28	0.20÷0.75=0.26
4.....	8.0−7.85=0.15	0.35	1.470	0.24	112	56	0.15÷0.75=0.20

more than doubled, but gradually decline. After 1 hour the relative rate of respiration was still above the normal. In this case the time of death could not be determined.

The experiments show that although the rate of respiration may be maintained for a time after death, it gradually falls off and eventually becomes very small. The question arises whether this falling off is due to exhaustion of the supply of oxidizable material or not. It is clear that when respiration has practically ceased there is a considerable amount of organic material left, but it is by no means certain that this material is such as to be easily oxidized by the ordinary processes which produce CO_2. On the

TABLE VIII

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 5 PERIODS (31.25 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL FINELY MINCED

Period	Change in PH	Change in PH after mincing = A	Change in PH before mincing, calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure after mincing	Relative rate of respiration
1.....	8.1−7.70=0.40	.....	.....	.....	31.25	.....	.....
2.....	8.1−7.70=0.40	.....	.....	.....	62.50	.....	.....
3.....	8.1−7.05=1.05	1.05	0.40	2.6	93.75	31.25	1.05÷0.40=2.6
4.....	8.1−7.65=0.45	1.50	0.80	1.9	125.00	62.50	0.45÷0.40=1.1
5.....	8.1−7.90=0.20	1.70	1.20	1.4	156.25	93.75	0.20÷0.40=0.5

other hand, we must consider the possibility that the production of CO_2 falls off because the supply of oxidizing enzymes is used up. Various observers have found that these enzymes may be used up (or inactivated) during oxidation (**1, 9**). If the process of oxidation involves the cooperation (or successive action) of various enzymes the inactivation of any one of them might bring the whole process to a standstill.

WARBURG (**21**), as the result of extensive study, has come to the conclusion that the rate of oxidation depends on the amount of "structure" which the cell possesses. If the "structure" is partially or completely destroyed the oxidation diminishes in propor-

tion, except in rare cases (as in the unfertilized sea urchin egg) where the oxidation is independent of structure. He states that the latter case disposes of the "reaction chamber" theory of cell structure, according to which the substances necessary for oxidation are separated by the semipermeable membranes of the cell in such a way as to regulate the speed of oxidation, for these substances can be completely mixed, as in the cytolysis of the unfertilized sea urchin egg, without any change in the rate of oxidation.

WARBURG'S treatment of the "reaction chamber" hypothesis seems to rest upon a misunderstanding. It is quite possible that in the cytolysis of the sea urchin egg the "reaction chambers" are not destroyed, since each of the fine granules into which the egg is resolved by cytolysis may be such a "reaction chamber" surrounded by a semipermeable surface.[4] In case some or all of the reaction chambers are destroyed by the treatment, because they are larger or for any reason more sensitive to the treatment, a change in the rate of oxidation may be expected (either an increase or decrease, according to circumstances). WARBURG himself states that where an increase of chemical action results from the injury the "reaction chamber" hypothesis seems to be justified. This is precisely what the writer finds. Increase of oxidation as the result of injury (although not as the result of death) has previously been recorded by many observers (**5**).

The "reaction chamber" hypothesis has much in its favor. An especially good example is the bitter almond, which at once produces HCN upon injury. In this case the reacting substances are known and we cannot escape the conclusion that previous to injury they fail to react because they are kept apart by structures in the cell. In some cases the mingling of substances, owing to the breaking down of such separating structures, can distinctly be seen under the microscope. This is the case with the marine alga *Griffithsia*, as described by OSTERHOUT (**14, 15**). When cells of this alga are injured by poisons (NH_4Cl), or mechanically, or by cytolysis with dilute sea water, the chromatophores (which contain a soluble red pigment) become permeable and the pigment can be seen passing

[4] The existence of an actual membrane is unnecessary.

out into the surrounding cytoplasm. It would seem, therefore, that in the absence of a better explanation[5] the reaction chamber hypothesis might serve a useful purpose.

Summary

The respiration of *Laminaria* after death may be considerably greater than in its normal condition. This is the case when it is killed by alcohol, acetone, formaldehyde, and ethyl bromide, as well as by drying and by other methods.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

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[5] The explanation suggested by WARBURG (**21**), that the oxidizing substances are bound up by the structure, seems too vague for discussion.

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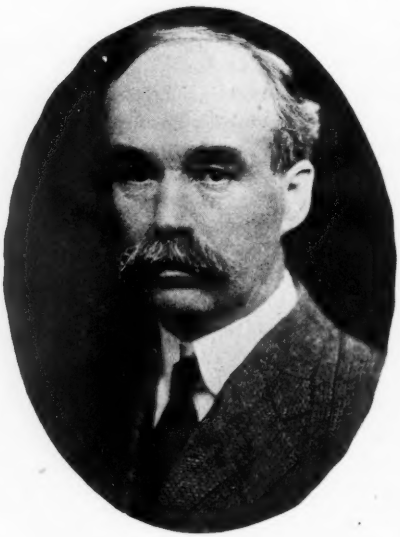
BRIEFER ARTICLES

GEORGE FRANCIS ATKINSON

(WITH PORTRAIT)

In the death of GEORGE FRANCIS ATKINSON on November 14, 1918, America lost one of her great botanists. Born in the little village of Raisinville, Monroe County, Michigan, on January 26, 1854, he received his preliminary collegiate training in Olivet College in that state. From there he went to Cornell University, where he took the degree of Ph.B. in 1885. Immediately upon graduation he became assistant professor of general zoölogy at the University of North Carolina. The following year he was made associate professor, remaining there until 1888, when he was called to a full professorship in botany and zoölogy in the University of South Carolina. In 1889 he was appointed professor of biology and botany in the Alabama Polytechnic Institute, where he remained until 1892. In 1892 he was called to Cornell University as assistant professor of botany, became associate professor in 1893, and upon the death of Professor Prentiss in 1896 was made full professor and head of the department. He was also for many years the botanist of the Cornell Agricultural Experiment Station. He continued head of the Department of Botany in the Arts College in Cornell University until his death.



Upon the request of an organization of his former students, the Board of Trustees of the University in 1917 relieved him of all teaching

and administrative burdens in order that he might devote his entire time and energies to the completion of his monographic studies on the fleshy fungi of North America.

In the vigorous and enthusiastic pursuit of this enterprise he made an extensive collecting trip through the Atlantic seaboard states from Florida to the District of Columbia in the spring and summer of 1918. Returning to Ithaca in September he left after an all too short rest for the Pacific Coast, there to pursue his studies of the fleshy fungus flora of that region. On this trip he was without any assistant and most of the time alone. A former student, Dr. ADELINE AMES, spent a few days collecting with him in the region about Tacoma, Washington, shortly before his death.

Urged by the wonderful variety and abundance of the forms he found and an indomitable enthusiasm for his work, he apparently labored beyond his strength and exposed himself to unusual hardships. He took a heavy cold from exposure on a trip into the mountains near Tacoma, Washington, which rapidly developed into influenza followed by pneumonia. He died in the City Hospital at Tacoma far from friends and kindred, another martyr to the cause of botanical science.

Professor FRYE of the University of Washington upon news of his death went immediately to Tacoma to learn the details and to rescue his notes and collections. Dr. AMES also went again to Tacoma shortly thereafter. Thanks to their generous and painstaking efforts we have a full account of Professor ATKINSON's last days. This record gives us a wonderful insight into the man's devotion to his work and a fuller appreciation of his greatness.

Interested primarily in entomology in the early days of his career, he soon turned to the botanical field, and especially mycology, in which perhaps he has made his most notable investigations. He was without doubt the greatest American student of the fleshy Basidiomycetes. His numerous contributions in this field and a remarkably large and exceptionally excellent collection of photographs, together with specimens and notes on these forms not only American but European attest his preeminence in this field. He was, however, a botanist of wide interests, and his investigations and writings touch nearly every branch of this broad field. A true philosopher, he gave to his contributions that philosophical character and flavor which is the mark of scientific genius.

He was the author of many textbooks, notable among which are several elementary and college textbooks of general botany, "The biology of ferns," and "Mushrooms, edible, poisonous, etc." He made many

contributions to the botanical journals, not only of America but also of England, France, and Germany.

His travels in Europe, his extensive correspondence, and the students that came from the ends of the earth to study in his laboratories have made his name familiar in the botanical institutions of every land. As a delegate to the International Botanical Congresses of 1905 and 1910 held in Vienna and Brussels respectively, he made for conservatism in botanical nomenclature. A charter member of the Botanical Society of America and at one time president, he has been for a generation one of the leaders of American botanical thought and activity. He was a fellow of the American Association for the Advancement of Science, a member of the American Philosophical Society, and in 1918 was elected to the National Academy of Science. He was for years an associate editor of the BOTANICAL GAZETTE. He was also a member of the honorary societies of Phi Beta Kappa and Sigma Xi.

To those of us who knew him intimately as teacher and friend, our days with him in field and laboratory will ever remain a happy and a grateful memory. He was a master of the highest scientific ideals, unsparing in his criticisms, just and fair in his judgments, generous with help and suggestions, a good friend and a genial companion.—H. H. WHETZEL, *Cornell University, Ithaca, N.Y.*

CURRENT LITERATURE

BOOK REVIEWS

Manual of tree diseases[1]

This first wholly American work in book form on the diseases of forest trees is one of "The Rural Manuals" edited by L. H. BAILEY, and in conformity with the general plan of this series has been written primarily for the general public. Insect and other animal injuries are not included. The treatment of the subject throughout is simple and direct; the diseases are concisely described, and methods of control indicated. The first 4 chapters deal with such maladies of biotic and abiotic origin as are common to many kinds of trees and are respectively entitled "Seedling diseases and injuries," "Leaf diseases and injuries," "Body and branch diseases and injuries," and "Root diseases and injuries." Chapters v–xxxii are devoted to an account of the more "specific" diseases, one chapter to each generic host group, beginning with the alders. The arrangement of the chapters is alphabetical according to the English host group names. Two chapters follow, one on "Tree surgery," the other on "Spraying and dusting for leaf diseases." The book is equipped with a glossary, a general bibliography of tree diseases, and an excellent index.

This work, although not intended as a textbook, will be welcomed by all students of plant pathology because it is the only summary available of the diseases of the forest trees of the United States and of Canada, and because it includes many classified references to the literature. The writing of the book reveals the limitations of forest pathology in America; the number of workers in this field has been small, the subject matter is as yet largely unexplored, and the applications of the results so far attained have been restricted. The author clearly recognizes these facts, and does not fail to point out the direction investigations should follow; in so doing he makes a contribution of prime importance.—J. H. FAULL.

MINOR NOTICES

Our national forests.—The period of reconstruction not less than the progress of the war has directed, in a special manner, the attention of our people to their natural resources and to the desirability of properly utilizing and conserving them. Thus no more timely moment could be chosen for the publication of some account of our forest wealth as shown in the establishment and

[1] RANKIN, W. HOWARD, Manual of tree diseases. pp. 398. *figs.* 70. 1918. New York: Macmillan Co.

management of our national forests. BOERKER[2] has collected and organized a mass of scattered data and presented them in a very readable form. While particularly well suited in its style of presentation to appeal to the general public, it will prove equally welcome to foresters and botanists who wish to know the history of the organization of these forests and the different forms of administration under which they have attained their present dimensions. The addition of a bibliography would have added much to the scientific value of the volume without detracting from its popular interest. It may also be criticized because of the lack of a suitable index to facilitate reference; but on the whole the work is well done, the material has been well organized, is attractively presented, and so far as the reviewer is able to judge the data are entirely accurate and reliable.—GEO. D. FULLER.

Grasses and grasslands of South Africa.—In order to facilitate the study of the extensive grasslands of South Africa, BEWS[3] has prepared a series of keys for the identification of the 500 species of grasses which form so conspicuous a portion of the flora of that part of the continent. These keys seem to be well suited to serve the purpose for which they are intended, but the other parts of the volume are of far more interest to the American reader. In them are discussed: (1) the structural and ecological characteristics of the principal species; (2) general character of the grasslands and the development of the various association types; and (3) economic application of the ecological principles involved. It is interesting to find types comparable to the "short grass," "wire grass," and "prairie grass" of North America, as well as a tall coarse *Andropogon* association, this last developing upon potential woodland areas, and a mountain tussock grassland. The discussion of the successional relations of these and other association types into which grasses enter gives a comprehensive general sketch of the plant communities of the major portion of South Africa.

In the final chapter the feeding value of the different types of grassland, as well as the comparative merits of native and introduced species, is discussed. The effect upon the productivity of various types of grassland by various kinds of grazing and the results from grass burning are considered and some of the ecological problems involved are pointed out. An appendix contains a list of English, Dutch, Zulu, and Sesuto names of the more important species.—GEO. D. FULLER.

NOTES FOR STUDENTS

Vegetation of Cape Breton.—Separated from the mainland of Nova Scotia by a narrow strait, the island of Cape Breton lies between the Gulf of St. Lawrence and the Atlantic in latitude 45–47° N. It possesses a climate

[2] BOERKER, RICHARD, H.D., Our national forests. pp. lxix+238. *figs. 80.* 1918. New York: Macmillan Co.

[3] BEWS, J. W., The grasses and grasslands of South Africa. 8vo. pp. 161. *figs. 24. map.* Pietermaritzburg: Davis & Sons. $2.00 (postpaid from author).

characterized by long winters and short cool summers, the extremes of temperature being modified by the close proximity of the ocean. A rainfall of 50 in. per year and frequency of fogs make the water supply sufficient for a luxuriant vegetation, which has been carefully studied by NICHOLS.[4] He finds two climatic forest formations represented, the deciduous type upon the lowlands which fringe the coast, and the coniferous type upon the granite uplands which occupy the entire interior portion of the island. These are about 1000 ft. above sea level and form a slightly undulating glaciated surface.

The lowlands show many associations depending upon the stage of development attained, and these variations and the successive stages which have led to their development are carefully discussed and the climax shown to be a forest dominated by beech, sugar maple, and hemlock, together with small quantities of *Betula lutea*, *Picea canadensis*, and *Abies balsamea*. The abundant details of these studies cannot be noticed in a brief review, but two problems in the relationship between the deciduous and evergreen elements of the vegetation are decidedly interesting. It has been found that upon the destruction of the deciduous forest by culling or burning it is succeeded by a coniferous stand dominated by *Abies balsama* and *Picea canadensis;* and further that the climax deciduous forest possesses a very considerable percentage of small *Abies balsamea* which never seem to succeed in competition with the other tree members of the association. NICHOLS presents evidence showing that the balsam fir is fairly shade tolerant, and that its lack of success is due to its short life, maturity being attained in about one century, and to its great susceptibility to fungus diseases.

It seems evident that the coniferous forest dominated by *Abies* and *Picea* is the climatic rather than the edaphic climax of all portions of the island exceeding 700 ft. in elevation. The factors which appear to differentiate the climate of the uplands from that of the lowlands are the greater extremes of temperature and the greater humidity due to fogs and low-hung clouds which frequently envelop the more elevated areas.

This upland forest is of decided importance in the production of pulp wood, its contents being estimated at 12,000,000 cords. Upon the more exposed parts of the uplands are developed "the barrens," closely resembling the tundras of the subarctic. The low vegetation of "the barrens" varies from a degenerate coniferous forest of the Krummholz type, where the distorted trees are limited in height to the thickness of the snow cover, to coniferous and ericaceous heaths, and to bogs of varied character. These bogs occupy considerable portions both of the lowlands and "the barrens," their most striking form being the raised peat bogs of the latter region, which have received careful attention, so that many problems connected with their development have been elucidated.

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[4] NICHOLS, GEO. E., The vegetation of northern Cape Breton Island, Nova Scotia. Trans. Conn. Acad. **22**:249–267. *figs.* 70. 1918.

NICHOLS finds the bogs of the raised type, corresponding to the "Hochmoors" of Europe, occurring upon this continent in Newfoundland and those parts of eastern Canada and Maine which are in close proximity with the sea coast. The climatic factors necessary for their development are abundant precipitation, relatively low atmospheric humidity, cool summers, and the absence of extremely low temperatures such as prevail farther inland. One of the necessary edaphic factors is an impervious substratum, here furnished by the Laurentian rocks. This is vitally important, since the source of water supply is the rainfall and not springs, as some have assumed.

The early stages of these raised bogs are not essentially different from those obtaining in bogs of the more common and familiar type, but their subsequent development is dependent upon the presence of distinct types of *Sphagnum*. NICHOLS classifies these mosses into 5 ecological groups, beginning with the decidedly aquatic and ending with those of comparatively xerophytic habits. It is upon the growth of the mesophytic and xerophytic sphagnums that the development of the dry raised bog depends. These mosses are cushion-forming in habit, and their successive development elevates the central portions of the bog many feet above its rim. Such a raised bog presents a hummocky surface, and except in wet weather a rather firm springy substratum quite dry underfoot. Upon the surface in addition to the xerophytic *Sphagnum* are other mosses such as *Racomitrium* and *Polytrichum*, some fruticose lichens, and several ericaceous shrubs for the most part less than a foot high. Scattered and dwarfed specimens of *Larix* and *Picea mariana* also occur. A typical specimen of the former, scarcely a foot high, possessed a trunk 1 inch in diameter showing more than 50 annual rings.

Another striking feature of the region seems to be the development of subsequent ponds within the bog area. These differ decidedly from the marginal trenches described by STALLARD,[5] which are due to fire consuming the peat in the shallow marginal portions of the bog during periods of unusual drought. The ponds in the Cape Breton bogs are due to the impervious nature of the peat from some of the sphagnums forming barriers and dams which obstruct the drainage on gentle slopes. Such ponds function as storage reservoirs, retaining much of the water which accumulates in them during wet periods and thus insuring to adjacent areas a constant supply throughout the season.

The development of the raised bogs, the subsequent bog ponds, and other features of the vegetation are illustrated by diagrams and photographs. The various successions are carefully traced and clearly described, the various communities being classified according to the system already noted.[6] In its comprehensive character, its abundance of detail, and its notable contributions to various phases of ecology, including the relationships between deciduous and coniferous forests, the ecology of the sphagnums and of the development of

[5] STALLARD, HARVY, The origin of *Sphagnum* atolls. New Phytol. **15**:250–256. 1918.

[6] BOT. GAZ. **66**:385–388. 1918.

raised bogs, this report stands as one of the most notable of recent years.—GEO. D. FULLER.

A new fixative for paraffin sections.—Dr. KOLOMAN SZOMBATHY[7] describes a new method of fixing paraffin sections to the slide. The fixative is claimed to have the advantage of not being dissolved by alkaline stains, and furthermore in not being stained by hematins and aniline stains such as eosin fuchsin, orange G., etc. The formula given by him is as follows: gelatin 1 gm., distilled water 100 cc., salicylate of soda (a 2 per cent solution) 1 cc., pure glycerine 15 cc.

Dissolve the gelatin in water at 30°, add the salicylate of soda, shake well, cool, and filter. To this add 15 gm. of pure glycerine. The solution obtained should be perfectly clear. A small amount of the fixative together with a drop or two of a 2 per cent formalin solution is placed on the slide, smeared evenly over the surface, and rubbed in well. Care should be observed that the formalin is mixed with the fixative. The sections or paraffin ribbons are then placed on the fixative and permitted to dry in the thermostat or any other warm place which is protected from dust. The formalin "tans" the gelatin and makes it insoluble. A modification of the method consists in exposing the slides, which have been mounted without the use of formalin solution, to vapors of concentrated formalin in a thermostat. The effect of the formalin is identical. A third method consists in preparing a solution of equal parts of 1 per cent gelatin in water and 2 per cent formalin. The fixative is then used as recommended for albumen fixative.

The writer has tested the fixative recommended by SZOMBATHY and finds it to be an excellent one. Material known to be difficult to retain on the slide was tried out. Sections of grass leaves and moss archegonia adhered to the slide even when the latter were left in running water for several days or exposed to a strong solution of hydrogen peroxide. Alkaline stains do not dissolve the gelatin nor do the stains tested stain the background to an appreciable extent.

Of the 3 methods originally recommended, the following modification gives the most satisfactory results. Make up the fixative according to the first formula, put a drop on the slide, and smear it evenly over the surface. Float the paraffin ribbon on the slide on a 2 per cent formalin solution. Warm the slide gently on the usual copper plate and, after the ribbon has straightened and become smooth, drain off the surplus water and let the preparation dry. When one is dealing with material which does not stick to the slide easily, it will be found of advantage to put a small dish of formalin in the thermostat where the preparations are drying, since the formalin vapors help in rendering the gelatin insoluble.

This new fixative is very easily prepared, keeps well, and does hold the sections to the slide. It should come into general use especially for material which does not adhere to the slide under ordinary conditions and when stains

[7] SZOMBATHY, KOLOMAN, Neue Methode zum Aufkleben von Paraffinschmitten. Zeitschr. Wiss. Mikr. **34**:334–336. 1918.

are employed which are alkaline in nature and have the objection of staining the background.—ERNEST F. ARTSCHWAGER.

Size variation in secondary xylem.—BAILEY and TUPPER[8] have applied the comparative method in thoroughgoing fashion to an attack on the problem of cell size. Confining themselves to a study of the length of the tracheary elements in the secondary xylem of trees and shrubs among vascular cryptogams, gymnosperms, and angiosperms, they present data derived from thousands of measurements on some 440 species belonging to 124 families. The most conspicuous fact brought out by this reconnaissance survey is that the length of these elements is roughly correlated with phylogenetic position, being greatest in vascular cryptogams, somewhat less in gymnosperms, and least in angiosperms. This progressive reduction in the length of the wood cells has been associated with the reduction in amount of the primary xylem in the passage from lower to higher forms, but is probably due in greatest measure to the evolution and differentiation of vessels. These elements have become progressively shorter and broader, thus losing their resemblance to the primitive tracheid; and the fibers and tracheids associated with them have also grown shorter, although naturally to a less extent. Notable exceptions to the general rule are the vessel-less Magnoliaceae and Trochodendraceae, represented by *Drimys* and *Trochodendron*, which possess tracheary elements far longer than other angiosperms, and thus resemble the gymnosperms. Evidence from this source obviously supports the view that these genera are primitive rather than reduced types.

The authors have also made a preliminary study of the relations between the length of the tracheary elements and the age of the plant, its growth habit, and the environment under which it lives. So far as the cells studied are concerned, there is no definite correlation between body size and cell size. The tracheary elements may increase in length for a few years as the plant grows larger, but they soon reach a constant size. Dwarfed and depauperate plants tend to have somewhat smaller elements than normal individuals.

The authors point to the need of more intensive investigations in this hitherto almost unexplored field; and in particular call for a careful study of the activities of the cambium and the factors which direct these activities. Indeed the growing point of plants, once so enthusiastically studied as the key to histology and then for so long neglected, bids fair to be once more a center of interest as one of the keys to a knowledge of morphogenesis.—E. W. SINNOTT.

Sap concentration in epiphytes.—Continuing the studies already noted[9] upon the concentration of tissue fluids, HARRIS[10] has found in several species of

[8] BAILEY, I. W., and TUPPER, W. W., Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms, and angiosperms. Proc. Amer. Acad. **54**:149–204. *figs. 6*. 1918.

[9] BOT. GAZ. **65**:285–286. 1918.

[10] HARRIS, J. ARTHUR, On the osmotic concentration of the tissue fluids of desert Loranthaceae. Mem. Torr. Bot. Club **17**:307–315. 1918.

Phoradendron growing in the Arizona deserts upon various hosts, such as species of *Acacia*, *Quercus*, *Fraxinus*, and *Populus*, that the osmotic concentration of the tissue fluids of the parasite is generally greater than that of the host. The concentration of the fluids of such parasites in this semidesert region is also greater than and usually about twice as great as that of similar plants found in the mountain rain-forests of Jamaica. These results quite agree with our expectations, but in a further paper the same investigator[11] clearly demonstrates the errors that would be involved in generalizing broadly on insufficient data.

The later investigations have to do with the tissue fluids of epiphytic Bromeliaceae, Orchidaceae, Piperaceae, and Gesneraceae, and these are shown to possess a decidedly lower concentration than those from terrestrial vegetation. In the mountain rain-forests of Jamaica the epiphytes show 37–60 per cent of the concentration commonly found in herbaceous terrestrial vegetation and 28–45 per cent of the concentration characteristic of ligneous soil plants. The epiphytes of the Jamaican rain-forests show lower concentrations than related plants of the same habit growing in the subtropical forests of Florida. The exactness of the data and quantitative character of the comparisons make these investigations important, and lead us to look forward for the further results promised in the study of parasitism by quantitative methods.—GEO. D. FULLER.

Bennettitales.—Two cones of the Bennettitales from the British Cretaceous, one of them a new species, have just been described by STOPES.[12] The first and most important is the one upon which she has founded the new species *B. albianus*, the specific name referring to the strata in which the specimen was found. Only a small piece of a single cone was found, but it was very well preserved. After a study of the topography, the entire fragment was cut, yielding 2 longitudinal and 5 transverse sections, the latter passing through the seeds and the former through their stalks. The most striking feature of the cone is its large size, not less than 70 mm. in diameter and probably more. The seeds are innumerable, as many as 600 showing in a single transverse section of the fragment. The seeds are 5–6 mm. long and 1.2 mm. in diameter, thus contrasting with the more or less ovoid seeds already described. The interseminal scales are fused around the apex of the seed. The embryo has 2 cotyledons and a rather massive hypocotyl and radicle.

The other specimen, *B. maximus*, was described from superficial characters by CARRUTHERS in 1870. The present study shows that the vascular axis is very small for such a large plant and the cones are bisporangiate, the first petrified bisporangiate cones which have been found in England. The cones

[11] HARRIS J. ARTHUR, On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. Amer. Jour. Bot. **5**:490–506. 1918.

[12] STOPES, MARIE C., New Bennettitean cones from the British Cretaceous. Phil. Trans. Roy. Soc. London **208**:389–440. *pls. 19–24.* 1918.

are very young but do not seem to have been well preserved. If material in this stage and somewhat older stages could be secured, it would help immensely in comparing the Bennettitales and the Cycadales.—C. J. CHAMBERLAIN.

Cytology of the basidium.—A cytological investigation of the basidium of *Eocronartium muscicola*, one of the Auriculariales parasitic upon mosses, was undertaken by FITZPATRICK[13] because he had noticed that the nuclei are of unusual size, and because very little cytological work has been done in this order. The mycelium, which is intracellular and extends throughout the host, is composed of binucleate cells. The cells of the sporophore are also binucleate, and, during division, it is seen that the number of chromosomes in each of the 2 nuclei is 4. During the development of the basidium, the 2 nuclei fuse, the resulting nucleus passes into synapsis, and in later stages of division shows 4 chromosomes, which is also the number at the second division, so that the total number of chromosomes in the cell is reduced. Toward the close of the second division a transverse wall appears in the middle of the basidium and is soon followed by two more walls, so that the basidium consists of a filament of 4 cells. The sterigmata, which are large in proportion to the cells from which they arise, are not quite simultaneous in their appearance. The chromatin becomes drawn out into a slender thread as the nuclei pass into the young spores, and there is no connection with the centrosomes, as has been reported for some basidia. How the binucleate mycelium arises from the uninucleate spore has not yet been determined.—C. J. CHAMBERLAIN.

Orientation of roots.—HOLMAN[14] has investigated the influence of the medium upon the orientation of primary terrestrial roots. He shows that the failure of roots grown in air to reach a vertical position is due to lack of mechanical resistance to the advance of the root tip after the flattening of the primary geotropic curvature, rather than to differences in water content in the medium, or changes in geotropic sensitiveness, or to thigmotropism. His observations have been extended to secondary roots,[15] and here also he finds that when they have been displaced from normal position with respect to gravity, and the first curvature of response has been flattened, mechanical resistance is necessary to a complete reaction to normal position. The mechanical resistance hinders flattening of the primary curvature of the root tip, and passively depresses the tip as it moves forward, thus reinforcing and completing the geotropic response. —C. A. SHULL.

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[13] FITZPATRICK, H. M., The cytology of *Eocronartium muscicola*. Amer. Jour. Bot. **5**:397–419. *pls. 30–32*. 1918.

[14] HOLMAN, RICHARD M., The orientation of primary terrestrial roots with particular reference to the medium in which they are grown. Amer. Jour. Bot. **3**:274–318. 1916.

[15] ———, Influence of the medium upon the orientation of secondary terrestrial roots. Amer. Jour. Bot. **3**:407–414. 1916.

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EFFECT OF ANESTHETICS UPON RESPIRATION

A. R. C. HAAS

(WITH SEVEN FIGURES)

The special interest which this subject has acquired, as the result of certain modern theories, makes it desirable to give a brief review of some of the more important contributions (**5, 10**) to our knowledge of it.

MEYER (**19**) and OVERTON (**21**) independently concluded that the effect of a narcotic increases with its solubility in substances of a lipoid nature. According to them narcosis does not appear until the lipoids of the cells have absorbed the narcotic to a definite molecular concentration (**21**). The theory has been criticized (**5, 10**) because it fails to explain why narcotics, such as benzamide and monacetin, which at higher temperature are less soluble in fat, have an effect which increases with the temperature. Both MEYER and OVERTON recognize the fact that often there is no relation between the narcotic power of a substance and its relative solubility in oil; the partition quotient for isobutyl alcohol is about 180 times greater than for ethyl alcohol, but its power to cause narcosis is only about 6 times as great as that of ethyl alcohol. They state that such cases cannot be used as arguments against the lipoid theory in case the narcotic is not chemically indifferent, but has a special reaction affinity, as is the case, for example, with the basic narcotics.

VERWORN (**29**) has advocated the view that narcotics interfere with the oxygen carriers of the cell and render them incapable of

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[13] FITZPATRICK, H. M., The cytology of *Eocronartium muscicola*. Amer. Jour. Bot. 5:397–419. *pls. 30–32*. 1918.

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